

**The physiological effects of *Sargassum* beach coverage on three species of sea
turtle hatchlings**

by

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Sarah Milton, Department of Biological Sciences, and has been approved by all members of the supervisory committee. It was submitted to the faculty of the Charles E. Schmidt College of Science and was accepted in partial fulfillment of the requirements for the degree of Master of Science.

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Abstract

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Sea turtle hatchlings face a variety of obstacles as they crawl down the beach to the ocean after emergence. One of these obstacles is *Sargassum*, a floating brown macroalgae, that washes up in large quantities on beaches from Florida to South America. This study examined the physiological response and physical performance of three species of sea turtle hatchlings (*D. coriacea*, *C. caretta*, and *C. mydas*) after crawling over various heights of *Sargassum*. In all three species, the addition of *Sargassum* significantly increased the amount of time it took to crawl down the pathway. There was no significant difference in righting response, blood glucose levels, or plasma corticosterone concentrations between different crawling treatments. During periods of high *Sargassum* accumulation, hatchlings will spend more time on the beach trying to navigate through the algae, leaving them vulnerable to predation for longer periods of time.

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Introduction

Florida has three different species of sea turtles that commonly nest on local beaches, including leatherbacks (*Dermochelys coriacea*), loggerheads (*Caretta caretta*), and green turtles (*Chelonia mydas*). After eggs are laid they incubate in the nest for an average of 50 to 80 days, depending on the species (Ackerman, 1997). Once incubation is complete the fully developed turtles must hatch and emerge from the nest. Emergence from the egg chamber consists of the hatchlings digging upward out of the nest, which marks the beginning of the frenzy period. The frenzy period is an extremely important stage for survival, and includes the hatchlings emerging from the egg chamber, crawling to the water, then actively swimming offshore (Carr and Ogren, 1960). This high level of activity makes it possible for hatchlings to move quickly offshore and escape high-predator areas as fast as possible (Wyneken and Salmon, 1992). Hatchlings swim continuously for approximately 24 hours during the frenzy period, so any added fatigue can lead to a decrease in their swimming capabilities (Booth, 2009) and hence their likelihood for survival.

Hatchlings rely on both aerobic and anaerobic metabolism during the energetically taxing frenzy period (Dial, 1987). Aerobic respiration occurs when there is oxygen available for electron transport within cells, allowing for quick and efficient ATP production. In times of extreme activity, anaerobic respiration

dominates and occurs when oxygen is not readily available to use; however, this process is not as efficient at producing ATP. Both aerobic and anaerobic respiration rely on glucose as a fuel source. An organism's aerobic scope is the difference between the maximum sustainable rate of oxygen consumption and the oxygen consumption rate at rest (Fry, 1947). Once the aerobic scope is exceeded a switch is made to anaerobic metabolism. This means that sufficient energy can no longer be produced through aerobic metabolism because the oxygen demand is too large (Bennett, 1978, 1972). While anaerobic metabolism may not be as efficient, it still permits energy to be produced so that physical activity can continue. Because the frenzy period is so energetically costly, hatchlings exceed their aerobic scope and utilize anaerobic metabolism during the digging, crawling, and swimming phases during this time (Baldwin et al., 1989; Pereira et al., 2012).

Glucose is the primary fuel source utilized by both aerobic and anaerobic metabolism and is released during high activity levels. As glycogen energy stores are depleted other fuel sources such as fatty acids and proteins are used (Hamann et al., 2007). This fuel shift will create an initial increase followed by a subsequent decrease in blood glucose during periods of intense exercise. Hatchlings have finite energy stores while moving offshore because they do not actively eat and instead rely on energy from their residual yolk (Kraemer and Bennett, 1981). Due to the energy limitations that occur during the crawling and swimming periods, any change in the amount of available fuel may impact their physical capabilities. A decrease in available energy stores while swimming may slow them down or prevent them from swimming as far, potentially making them

more vulnerable to predation (Hamann et al., 2007). Any added obstacles encountered during the frenzy stage will increase both their energy expenditure and stress response, resulting in an initial increase in both glucose levels and stress hormones, the latter of which partly control metabolism.

The hypothalamus-pituitary-interrenal axis is a main component of an organism's stress response, which results in the release of glucocorticoids (Moon, 1961). One glucocorticoid, corticosterone, is used by reptiles and allows energy demands to be met during stressful activities (Nelson, 2005). Increases in corticosterone mobilize energy stores such as glycogen, fat, and protein, which help to increase physical performance during times of stress or high activity by providing extra fuel for metabolism (McEwen and Wingfield, 2003). Stress hormones have also been suggested to aid in coordination and long distance migrations, activities similar to hatchling dispersal (Hamann et al., 2007). Corticosterone is found in various concentrations during different life-stage classes of sea turtles. In hatchlings, a corticosterone peak occurs immediately after digging out of the nest (Hamann et al., 2007; Pereira et al., 2012), which likely occurs to allow for increased performance during hatching and emergence (Morris, 1982) and predator avoidance. High corticosterone levels are linked to high-intensity activities, so turtles that undergo more stressful and physically demanding exercises will have increased corticosterone blood concentrations (Hamann et al., 2007). However, while necessary in the short term, chronically elevated levels of this stress hormone can have negative long-term impacts on physical performance and physiology by redirecting energy that would otherwise

be allocated for other processes, such as growth and immune response (Wendelaar Bonga, 1997; Milton and Lutz, 2003).

Along with affecting energy reserves, hatchlings face obstacles on their crawl down to the water (e.g., vehicle ruts, artificial lighting, marine debris, etc.) which may slow them down (Aguilera et al., 2019, 2018; Hosier et al., 1981; Pankaew and Milton, 2018; van de Merwe et al., 2012). Any added time on the beach increases their exposure to potential terrestrial predators, such as ghost crabs, foxes, raccoons, and birds (Erb and Wyneken, 2019), and may thus decrease their physical ability or stamina when they enter the water. One obstacle of growing concern is *Sargassum* that has been accumulating on beaches more frequently in recent years, which hatchlings must therefore traverse on their way from the nest to the water. *Sargassum* is a genus of floating brown algae found mainly in the Atlantic Ocean (Doyle and Franks, 2015) and is an important habitat for many marine organisms. Located in the North Atlantic Subtropical Gyre, the Sargasso Sea serves as an important refuge for sea turtles in their post-hatchling and early juvenile years. As a floating seaweed, *Sargassum* is carried by currents and is known to wash up on beaches, where it can help stabilize beaches and dunes and adds nutrients to the sand (Williams and Feagin, 2010). Therefore, there are benefits to *Sargassum* accumulation on beaches; however, in the last decade, *Sargassum* biomass has increased in oceans and on beaches (Schell et al., 2015; Wang and Hu, 2016), leading to some potential negative impacts within the beach ecosystem.

In 2011 the first big peak of *Sargassum* was recorded, washing up in large quantities on beaches from Florida to South America and reaching the coast of Western Africa. *Sargassum* mats wash ashore from spring-fall in large amounts during bloom years (Oviatt et al., 2019). This time period overlaps with sea turtle nesting season in Florida, which occurs on Florida's east coast during March – October. The accumulation of *Sargassum* could thus have a potential impact on the three sea turtle species that commonly nest in this location. In some areas of the Caribbean (e.g., Antigua), vast quantities of *Sargassum* washed ashore and negatively impacted hawksbill sea turtle activity when gravid females could not crawl over the piles of *Sargassum* to nest, which reduced the amount of viable nesting area by 25% (Maurer et al., 2015). Significant accumulations of *Sargassum* are expected to continue in coastal areas and may be related to climate change as well as nutrient input (Louime et al., 2017). Large amounts of nutrients are entering the ocean through the Amazon River, which coupled with warming water is most likely leading to this increase in *Sargassum* biomass (Wang et al., 2019).

There has been little investigation into the impact that crawling over *Sargassum* may have on sea turtle hatchlings. Schiariti and Salmon (2022), found that *Sargassum* was impassible by loggerhead hatchlings at a height of 30 cm and another study found that crawling over wrack zone seagrass (*Cymodocea nodosa*) increased crawl time in loggerhead hatchlings in the Mediterranean (Triessnig et al., 2012). An observational study of *Sargassum* in the Caribbean suggested that hatchlings will likely struggle crawling and swimming through dense mats of

Sargassum; however no quantitative data on this hypothesis has been reported to date (Maurer et al., 2019; 2015). Additionally, published data are lacking on physiological responses of hatchlings to crawling over *Sargassum* on the beach. Therefore, increasing amounts of *Sargassum* on beaches may pose both physical and physiological threats to sea turtle hatchlings during nesting season

This study examined the impact of *Sargassum* accumulation on three sea turtle species (leatherback, loggerhead, and green turtles) that commonly nest in Florida in an effort to identify similarities and differences in how the species react to *Sargassum* beach coverage. The three species differ in which time of season hatchlings emerge, mean nest temperature, and hatchling size and locomotor performance, which could all affect the impact the ability of hatchlings to navigate *Sargassum* piles successfully to reach the surf.

Hypotheses and Objectives

The purpose of this research was to assess the physiological and physical impact of *Sargassum* accumulation on beaches on sea turtle hatchlings crawling to the ocean after emergence. I hypothesized that:

H1: An increase in *Sargassum* in the crawl pathway will affect hatchling crawl time, righting response time, and blood glucose and plasma corticosterone concentrations.

H01: An increase in *Sargassum* in the crawl pathway will have no effect on these measures of physical and physiological performance.

H2: All three species (*D. coriacea*, *C. caretta*, and *C. mydas*) will follow a similar trend of altered crawl time, righting response time, blood corticosterone concentration and glucose levels as *Sargassum* increases.

H02: There will be differences between species in these measures of physical and physiological performance.

To test these hypotheses, I examined the following questions:

1. Is there a correlation between the time it takes the hatchlings to crawl through the treatment (piled *Sargassum*) and the height of the *Sargassum* in the pathway?
2. Is there a correlation between hatchling blood glucose and corticosterone concentrations post-crawl and the amount of *Sargassum* in the pathway?
3. Is there a correlation between hatchling righting response time post-crawl and the amount of *Sargassum* in the pathway?
4. Are there significant differences in the objectives listed above between *D. coriacea*, *C. caretta*, and *C. mydas*?

Methods

All research was conducted under FWC permit # MTP-21-053A and IACUC protocol #A21-26. Research methods complied with protocols in both permits.

Study Site and Species

This study was conducted on three beaches in Florida: Juno Beach (26.8798° N, 80.0534° W), Jupiter (26.9342° N, 80.0942° W), and Boca Raton (26.3683° N, 80.1289° W). Three species of sea turtle hatchlings were investigated, including leatherbacks, loggerheads, and green turtles. Leatherback hatchlings were collected from nests in Juno Beach and Jupiter that were monitored by Loggerhead Marinelife Center. Loggerhead and green turtle hatchlings were collected from nests located in Boca Raton that were monitored by Gumbo Limbo Nature Center.

Nests

A total of 30 nests were used, including 10 nests from each species and 10 hatchlings from each nest. Each of the nests had a temperature data logger in the egg chamber throughout the incubation period. In nests in Boca Raton, HOBO Temperature Pro v2 Data Logger, with an accuracy of $\pm 0.2^{\circ}\text{C}$ (Onset Computer Corp, Bourne, MA), was inserted into the clutch the morning after the nest was

laid (insertion occurred before 9AM). To do this, the top eggs were removed, the datalogger was inserted, and the eggs were placed back into the egg chamber in the order that they were removed. This was done by staff at Gumbo Limbo Nature Center. In nests in Juno Beach and Jupiter the HOBO Temperature Pro v2 Data Loggers were placed in the nest during oviposition in leatherback nests by Loggerhead Marinelife Center staff. The data loggers remained in the nests until emergence and recorded the temperature at 15- or 30-minute intervals throughout the incubation period.

Hatchling Collection

Hatchlings were collected during natural hatch outs at night. When a nest emergence was expected, a wire mesh cage was placed around the nest and monitored throughout the night. After emergence, the hatchlings were removed from the cage and placed in a bucket with warm, damp sand, which was then covered with a towel to keep them in the dark and quiescent until the crawling trial began. Trials began within 10 minutes of emergence. From each nest, 10 hatchlings were collected to include 2–3 hatchlings per treatment group (described below). Hatchlings that were selected were free from any noticeable deformities and were active after emergence. Twenty hatchlings were included in each of the four treatment groups for each species, totaling 80 hatchlings per species. Twenty extra hatchlings per species were collected as “spares” in each treatment group in case any hatchlings failed to perform or blood collection attempts were not successful. Each hatchling went through only one trial; no hatchlings were used for multiple treatments. All hatchlings were released into the

ocean on the night they were collected, once data collection was complete. A flowchart of the methods can be seen in Figure 1.

Crawl Pathway

Each hatchling crawled down a 15x1 m crawl pathway on the beach where they hatched, which was cleared of all debris and raked relatively smooth. Black plastic sheeting 6 inches tall lined either side of the runway to keep the hatchlings crawling in a fairly straight line down the beach towards the ocean and prevent misorientation. A dim light was also used to guide hatchlings down the pathway. The substrate of the first 13 m of the pathway consisted of only sand, and the last 2 m had varying amounts of *Sargassum* based on the treatment group. The *Sargassum* heights were selected to provide a comparison between two different amounts while ensuring that the maximum height was less than 30cm, the maximum height determined by Schiariti and Salmon (2022) that hatchlings could surmount. Fresh *Sargassum* was collected from the beach as needed to construct the runway, typically three times per week. In both the light and heavy *Sargassum* treatments, the hatchlings had to crawl up a $\sim 70^\circ$ angle to reach the top of the *Sargassum*.

The following treatments were used:

No Crawl Control: Hatchlings did not crawl down the beach path. All measurements were collected after emergence before crawling to the ocean.

Crawl Control: Hatchlings crawled 15 m down the beach pathway that was free of any *Sargassum* or other obstacles, crawling only on bare sand.

Light Coverage: Hatchlings crawled 15 m down the beach pathway with *Sargassum* at the end. The first 13 m of the crawl was on bare sand and the last 2 m was on a continuous patch of *Sargassum* 7–9 cm high (Schiariti and Salmon, 2022).

Heavy Coverage: Hatchlings crawled 15 m down the beach pathway with *Sargassum* at the end. The first 13 m of the crawl was on bare sand and the last 2 m was on a continuous patch of *Sargassum* 17–19 cm high (Schiariti and Salmon, 2022).

The order in which hatchlings were run through the different treatment groups was rotated each night, so that the same treatment was not first or last each time. Figure 2 depicts a schematic of the different treatment groups.

Times

As hatchlings crawled down the pathway, various times were recorded, as listed below:

Sand Time: Amount of time to crawl down the first 13 m of the crawl pathway over only sand.

Climb Time: Amount of time to crawl from the sand to the top of the *Sargassum* pile. This time started when hatchlings touched the *Sargassum* and ended when the hatchling's entire body was on top of the *Sargassum* pile. The crawl control group did not have this time category since they simply continued their crawl on sand only.

***Sargassum* Time:** Amount of time to crawl the final 2 m of the crawl pathway on top of the *Sargassum*. For the crawl control, these final 2 m were still on sand, but are referred to as the *Sargassum* time for comparison to the other treatment groups.

Total Time: Amount of time from the start to end of the crawl pathway (Sand Time + Climb Time + *Sargassum* Time).

Time Limits

Loggerhead and green turtle hatchlings were allowed to crawl down the pathway for a maximum of 30 total minutes, and leatherbacks for a maximum of 45 total minutes. Leatherbacks were given a longer time limit because they have a slower crawl speed than loggerhead and green turtle hatchlings (Seaman, 2020).

Blood samples

After each hatchling completed its crawl, a small volume of blood was collected to determine blood glucose and corticosterone concentrations. Prior to venipuncture, the skin over the external jugular vein area was disinfected using alternating swabs of povidone iodine and 70% isopropyl alcohol, and $\leq 100 \mu\text{l}$ of blood was collected with a sodium-heparinized, 1 ml (tuberculin) syringe fitted with a 1 cm 26-gauge needle. Blood was collected using safe practices (NOAA SEFSC, 2008). Blood samples were placed in a cooler on an elevated rack that ensured samples did not have direct contact with ice and transported back to FAU to be centrifuged then stored in a -80°C freezer. Samples were centrifuged for 10 minutes using a My Fuge Mini at 6,000 rpm. Hemolysis scores were also

recorded and samples with a score of 2 or higher were not used. Immediately after the blood was drawn and before the sample was placed in the cooler, one drop of blood was used to measure blood glucose with a standard blood glucose monitor (Easy Touch Blood Glucose Monitor, validated by Kunze et al., 2020; Perrault et al., 2018). Corticosterone was measured from blood plasma using the Corticosterone ELISA kit (ADI-900-097), methods followed kit instructions for plasma samples and as described in Henaghan (2018). It is important to note that the small volume protocols were used to run the CORT samples which involved a 1:40 dilution of the plasma with assay buffer.

Righting Response

Once the blood sample was collected, a righting response test was conducted in a 19 L bucket filled with ~10L of seawater. New seawater was collected directly from the ocean where trails were run; new seawater was collected for each nest. Hatchlings were placed upside down on their carapace and the time it took them to right themselves back onto their plastron was recorded with a stopwatch (Fleming et al., 2020). Each hatchling underwent three timed trials, and the average time was calculated.

Hatchling Size

Using handheld digital calipers, hatchling measurements were taken as soon as blood sample collection and the righting response test were completed, including straight carapace length (SCL), straight carapace width (SCW), flipper

length (FL), body depth, and mass. Body condition index (BCI) was calculated using the following equation: $(\text{mass}/\text{SCL}^3) \times 1000$ (after Bjorndal et al. 2000).

Air, Sand, and *Sargassum* Temperatures

As environmental temperature can impact reptile metabolism and activity (Davenport, 1997; Triessnig et al., 2012), air, sand, and *Sargassum* temperatures were recorded. Sand temperature was measured at the beginning, middle, and end of the crawl pathway and the average temperature was calculated. Air temperature was recorded using a weather app (Accuweather). All other temperatures were measured using a thermal gun (Fluke 62 Max Infrared Thermometer).

Statistical Analyses

All statistical analyses were conducted using R software (version 4.0.4). Significance was determined by a p-value >0.05 .

Pre-data collection analysis

A power analysis was conducted prior to data collection to determine the number of hatchlings needed for each treatment group, which was found to be 20 hatchlings per treatment for each species.

Post-data collection analysis

To determine if the data were normally distributed, QQ plots and Shapiro-Wilk's tests were used. All data were found to violate the assumptions of normality; therefore, Kruskal-Wallis tests were performed to determine significant differences between crawl times (sand time, climb time, *Sargassum* time, and

total time), glucose and corticosterone concentrations, righting response time, and temperatures between treatment groups and species. If statistically significant differences were found ($p < 0.05$), Dunn's nonparametric post-hoc comparisons were conducted.

Results

Overview

In all three species evaluated, the addition of *Sargassum* was associated with increased total time spent crawling down the pathway, particularly in the heavy *Sargassum* treatment group. Most of the extra time spent crawling occurred when hatchlings were climbing from the sand to the top of the *Sargassum* pile, during which time many hatchlings flipped over and had to right themselves. These effects increased as the height of *Sargassum* increased. Leatherback hatchlings were the slowest to crawl through the pathway, followed by loggerheads and green turtles (Table 1).

For all three species, blood glucose concentrations did not significantly differ between the different treatment groups. The only exception to this was that leatherbacks in the no crawl control group had significantly higher glucose concentrations than leatherbacks in any of the crawling treatment groups. For all three species, there was also no significant difference between treatment groups for corticosterone concentrations. There was no difference in post-crawl righting response within any species between the different treatment groups. Hatchling SCL and BCI did have some significant impact on crawl times depending on species and treatment group. Environmental temperatures (sand and *Sargassum*) did not have a significant impact on crawl times. In regard to nest temperature,

the only species that had significantly slower crawl times with warmer nest incubation temperatures were green turtles in some treatment groups, loggerhead and leatherback hatchlings did not have any significant correlations between incubation temperatures and crawl time.

Leatherbacks

Total Time

Hatchlings in the crawl control group required an average of 322 s to crawl the length of the pathway, the light *Sargassum* treatment required an average of 433 s, and the heavy *Sargassum* required an average of 1072 s. Hatchlings in the light *Sargassum* treatment had a 34.7% increase in total time when compared to the crawl control group, while those in the heavy *Sargassum* treatment group took 233.4% longer than the crawl control group (Table 1, Figures 3 and 4). The total time to crawl the entire length of the pathway was significantly different between all treatment groups, with *Sargassum* cover increasing the total time (Kruskal-Wallis: $p=5.88E-06$. Dunn's: crawl control-heavy $p=0$, crawl control-light $p=0.0197$, light-heavy $p=0.0229$). This was primarily due to the time it took hatchlings to crawl onto the *Sargassum* pile.

Table 1: Average and standard deviation of the different recorded crawl times in the pathway for each species. Significance is indicated with *, numbers with a different number of * are significantly different from each other for the same measurement. Significance shown only within species, not between the species.

Leatherback				
Treatment	Sand Time (s)	Climb Time (s)	<i>Sargassum</i> Time (s)	Total Time (s)
No Crawl	NA	NA	NA	NA
Crawl	266 (± 146)	NA	54 (± 25)	321.60 (± 163)
Light	244 (± 110)	34 (± 28)	158 (± 84) *	433.26 (± 138) *
Heavy	240 (± 101)	708 (± 905) *	145 (± 67) *	1072.35 (± 883) **

Loggerhead				
Treatment	Sand Time (s)	Climb Time (s)	<i>Sargassum</i> Time (s)	Total Time (s)
No Crawl	NA	NA	NA	NA
Crawl	168 (± 61)	NA	40 (± 16)	208 (± 76)
Light	167 (± 39)	86 (± 130)	213 (± 164) *	461 (± 267) *
Heavy	166 (± 75)	533 (± 648) *	160 (± 79) *	800 (± 627) *

Green Turtle				
Treatment	Sand Time (s)	Climb Time (s)	<i>Sargassum</i> Time (s)	Total Time (s)
No Crawl	NA	NA	NA	NA
Crawl	84 (± 27)	NA	25 (± 7)	109 (± 31)
Light	93 (± 26)	44 (± 123)	93 (± 45) *	230 (± 152) *
Heavy	94 (± 28)	246 (± 498) *	85 (± 28) *	416 (± 480) *

Sand Time

The amount of time to crawl only the sand portion of the crawl pathway (the first 13 meters) was not significantly different between treatment groups (Kruskal-Wallis: $p=0.9385$). (Table 1, Figure 5).

Climb Time

Hatchlings in the heavy *Sargassum* treatment group took 320.2% longer to climb onto *Sargassum* than hatchlings in the light treatment group (Table 1, Figure 6). There was a significant difference between treatments (Kruskal Wallis: $p=5.61E-07$). In the heavy *Sargassum* treatment the climb time portion of the crawl pathway greatly increased the total time.

***Sargassum* Time**

Compared to the crawl control group, hatchlings in the light *Sargassum* treatment group took 191.0% longer to complete the *Sargassum* portion of the crawl pathway, and hatchlings in the heavy *Sargassum* treatment group took 167.8% longer (Table 1, Figure 7). The light and heavy *Sargassum* treatment groups took significantly longer than the crawl control, but their times did not significantly differ from each other (Kruskal-Wallis: $p=9.97E-08$. Dunn's: crawl control-heavy $p=0$, crawl control-light $p=0$, light-heavy $p=0.974$).

Inversions

Heavy *Sargassum* cover was significantly associated with increased instances of hatchlings inverting. Both the crawl control and light *Sargassum* groups had significantly fewer inversions than the heavy *Sargassum* group but did not significantly differ from each other (Kruskal-Wallis: $p=1.83E-07$. Dunn's: crawl control-heavy $p=0$, crawl control-light $p=0.1325$, light-heavy $p=0.0003$) (Table 2; Figure 8). Along with the differences in inversions between treatments, both total time and *Sargassum* climb significantly increased as number of

inversions increased (linear regression: light $p=0.04412$, $R^2=0.1624$, heavy $p=2.58E-06$, $R^2=0.7003$) (Figure 9).

Table 2: Average and standard deviation for number of inversions while in the crawl pathway, glucose, corticosterone (CORT), and righting response. Significance is indicated with *, numbers with a different number of * are significantly different from each other for the same measurement. Significance shown only within species, not between the species.

Leatherback				
Treatment	Inversions	Glucose (mg/dl)	CORT (pg/ml)	Righting Response (s)
No Crawl	NA	115.2 (\pm 34.23)	76.33 (\pm 63.61)	1.38 (\pm 0.41)
Crawl	0 (\pm 0)	94.52 (\pm 24.11) *	42.45 (\pm 20.09)	1.26 (\pm 0.23)
Light	0.65 (\pm 1.14)	93.85 (\pm 23.49) *	47.12 (\pm 15.89)	1.45 (\pm 0.55)
Heavy	6.35 (\pm 8.79) *	92.25 (\pm 29.29) *	45.14 (\pm 21.78)	1.52 (\pm 0.29)

Loggerhead				
Treatment	Inversions	Glucose (mg/dl)	CORT (pg/ml)	Righting Response (s)
No Crawl	NA	131.95(\pm 51.69)	281.45 (\pm 93.75)	0.88 (\pm 0.28)
Crawl	0 (\pm 0)	120.45 (\pm 31.41)	250.59 (\pm 85.50)	0.79 (\pm 0.16)
Light	0.9 (\pm 1.65)	115.11 (\pm 29.08)	206.94 (\pm 117.60)	0.77 (\pm 0.17)
Heavy	4.05 (\pm 7.84) *	117.05 (\pm 25.2)	198.95 (\pm 92.49)	0.88 (\pm 0.24)

Green Turtle				
Treatment	Inversions	Glucose (mg/dl)	CORT (pg/ml)	Righting Response (s)
No Crawl	NA	140.79 (\pm 36.84)	192.54 (\pm 96.06)	0.64 (\pm 0.15)
Crawl	0 (\pm 0)	120.45 (\pm 40.60)	176.69 (\pm 20.09)	0.61 (\pm 0.11)
Light	0.7 (\pm 2.23)	112.85 (\pm 29.53)	160.48 (\pm 72.22)	0.71 (\pm 0.23)
Heavy	3.6 (\pm 6.57) *	126.46 (\pm 41.81)	107.93 (\pm 109.45)	0.66 (\pm 0.12)

Time Limit

Four hatchlings were not able to crawl the length of the entire pathway within the 45-minute time limit. Each of these hatchlings crawled through the sand portion successfully; however, when they reached the *Sargassum*, they were not able to climb up and over to the end of the pathway.

Blood

Glucose

Blood glucose concentrations in the no crawl control were significantly higher than all other treatment groups (Table 2, Figure 10). This was the only significant difference found between groups (Kruskal-Wallis: $p=0.007511$. Dunn's: crawl control-heavy $p=1$, crawl control-light $p=1$, crawl-no crawl $p=0.0125$, light-heavy $p=1$, heavy-no crawl $p=0.0154$, light-no crawl $p=0.0159$). All crawling trials had similar glucose concentrations.

Corticosterone

Blood plasma corticosterone concentrations were not significantly different between treatment groups (Kruskal-Wallis: $p=0.2119$) (Table 2, Figure 11).

Righting Response

There were no significant differences between righting response in any of the treatment groups (Kruskal-Wallis: $p=0.1876$) (Table 2, Figure 12).

Hatchling Size

Mean SCL, SCW, FL, body depth, mass, and BCI are summarized in Table 3. In the crawl control treatment longer SCL significantly correlated with slower total crawl times (Figures 13 and 14), all other treatment groups were not significant. SCW had no significant correlations to crawl times (Table 4, Figures 15 and 16). Flipper length had no significant correlations to crawl times (Table 4, Figures 17 and 18). Deeper body depth significantly correlated with slower total crawl time in the light *Sargassum* treatment, but all other correlations were not significant (Table 4, Figures 19 and 20). Mass was not significantly correlated to crawl times (Table 4, Figures 21 and 22). Body Condition Index was marginally correlated, with higher BCI correlating to faster total crawl times and climb times in the crawl control and heavy *Sargassum* treatment groups (Table 4, Figures 23 and 24); all other treatment groups were not significantly correlated.

Table 3: Average and standard deviation for various hatchling size measurements. Significance is indicated with *, numbers with a different number of * are significantly different from each other for the same measurement.

Species	SCL (mm)	SCW (mm)	FL (mm)	Depth (mm)	Mass (g)	BCI (g/mm ³)
Leatherback	59.55 (± 2.15)	40.50 (± 2.26)	58.47 (± 2.15)	23.93 (± 1.31)	44.88 (± 3.23)	2.13 (± 0.18)
Loggerhead	43.42 (±2.13) *	32.54 (± 1.77) *	28.44 (± 2.32) *	16.45 (± 1.59) *	18.08 (± 2.23) *	2.21 (± 0.17)
Green Turtle	49.46 (± 2.38) **	36.89 (±1.91) **	36.45 (± 2.13) **	17.45 (± 1.73) **	24.05 (± 1.89) **	2.00 (± 0.27) *

Table 4: Linear regression statistical output for hatchling body size and crawl times. Significance indicated by * and bold.

SCL-total time			SCW-total time			FL-total time		
	p-value	R ²		p-value	R ²		p-value	R ²
Crawl	0.01109*	0.2695	Crawl	0.4053	-0.01462	Crawl	0.4446	-0.02091
Light	0.212	0.03428	Light	0.4617	-0.02339	Light	0.7933	-0.05142
Heavy	0.2081	0.03577	Heavy	0.7291	-0.04835	Heavy	0.9206	-0.05496
SCL-climb time			SCW-climb time			FL-climb time		
Light	0.9686	-0.05546	Light	0.62	-0.04084	Light	0.7006	-0.04668
Heavy	0.2926	0.00915	Heavy	0.5337	-0.03246	Heavy	0.7777	-0.05076

Body depth-total time			Mass-total time			BCI-total time		
DC	p-value	R ²	DC	p-value	R ²	DC	p-value	R ²
Crawl	0.4930	-0.02759	Crawl	0.5308	-0.03214	Crawl	0.0098*	0.2780
Light	0.0268*	0.2119	Light	0.1149	0.08411	Light	0.8394	-0.0530
Heavy	0.6468	-0.04297	Heavy	0.8993	-0.05459	Heavy	0.05481	0.1448
Body depth-climb time			Mass-climb time			BCI-climb time		
Light	0.4740	-0.0251	Light	0.5456	-0.03377	Light	0.6752	-0.0450
Heavy	0.4921	-0.02748	Heavy	0.7142	-0.0475	Heavy	0.05567	0.1436

Temperature

Sand

Mean sand temperature was 25.8°C ($\pm 1.1^\circ\text{C}$) with a range of 23.7–27.9°C. There was no significant linear correlation between sand temperature and crawl time (linear regression for sand time: crawl control $p=0.9391$, $R^2=-0.0552$, light $p=0.6851$, $R^2=-0.04569$, heavy $p=0.6842$, $R^2=-0.04563$. Linear regression for total time: crawl control $p=0.9897$, $R^2=-0.0555$, light $p=0.4153$, $R^2=-0.0163$, heavy $p=0.3277$, $R^2=0.000642$) (Figure 25 and 26).

Sargassum

Mean *Sargassum* temperature was 26.5°C ($\pm 1.3^\circ\text{C}$) with a range of 23.3–30.3°C. There was no significant linear correlation between *Sargassum* temperature and crawl times (linear regression for climb time: light $p=0.3006$, $R^2=0.007703$, heavy $p=0.5614$, $R^2=-0.03542$. Linear regression for *Sargassum* time: light $p=0.2233$, $R^2=0.03213$, heavy $p=0.3571$, $R^2=-0.006252$) (Figures 27 and 28).

Nest

Mean nest temperatures are outlined in Table 5. Nest temperature did not have a significant correlation with total time or *Sargassum* climb time (Table 6). This is true for the overall mean nest temperature during the entire incubation period, the maximum temperature recorded, and the highest consecutive three-day mean temperature (Figure 29, 30, 31, 32, 33, and 34).

There was no significant difference between nest temperatures and blood plasma corticosterone concentrations (Figures 35-37).

Table 5: Average and standard deviation for various nest temperatures for each species. Significance is indicated with *, numbers with a different number of * are significantly different from each other for the same measurement.

Species	Mean Daily Temperature (°C)	Mean Max Temperature (°C)	Mean Three-Day Max Temperature(°C)
Leatherback	30.85 (± 0.58) *	33.92 (± 1.08) *	33.56 (± 0.84) *
Loggerhead	32.55 (± 0.63)	35.32 (± 0.63)	34.88 (± 0.61)
Green Turtle	32.72 (± 0.58)	35.27 (± 0.56)	34.80 (± 0.63)

Table 6: Linear regression statistical output for nest temperature correlation to crawl times. There were no significant correlations found.

Mean nest temp-total time			Mean nest temp-climb time			Max nest temp- total time		
	p-value	R ²		p-value	R ²		p-value	R ²
Crawl	0.2529	0.02036	Light	0.772	-0.0505	Crawl	0.4208	-0.0172
Light	0.7336	-0.0486	Heavy	0.4599	-0.02314	Light	0.9088	-0.05476
Heavy	0.5679	-0.03607				Heavy	0.9838	-0.05553

Max nest temp-climb time			3 day max nest temp-total time			3 day max nest temp-climb time		
	p-value	R ²		p-value	R ²		p-value	R ²
Light	0.5618	-0.03546	Crawl	0.5864	-0.03786	Light	0.6152	-0.04043
Heavy	0.9121	-0.05482	Light	0.9371	-0.05518	Heavy	0.5313	-0.03219
			Heavy	0.6675	-0.04449			

Loggerheads

Total Time

Hatchlings in the crawl control group required an average of 208 s to crawl the length of the pathway, the light *Sargassum* treatment required an average of 461 s, and the heavy *Sargassum* required an average of 800 s. Hatchlings in the light *Sargassum* treatment had a 121.8% increase in total time when compared to the crawl control group, while those in the heavy *Sargassum* treatment group took 284.6% longer than the crawl control group (Table 1, Figures 3 and 4). The light *Sargassum* and heavy *Sargassum* treatments were both significantly different than the crawl control, however they were not significantly different from each other (Kruskal-Wallis: $p=4.46E-07$. Dunn's: crawl control-heavy $p=0$, crawl control-light $p=0$, light-heavy $p=0.2262$). The addition of *Sargassum* did increase the total time spent in the pathway. This was primarily due to the time it took hatchlings to crawl onto the *Sargassum* pile.

Sand Time

The amount of time to crawl on the sand portion of the crawl pathway (i.e., the first 13 m) was not significantly different between treatment groups (Kruskal-Wallis: $p=0.8585$) (Table 1, Figure 5).

Climb Time

Hatchlings in the heavy *Sargassum* treatment group took 518.5% longer to climb onto the *Sargassum* than hatchlings in the light treatment group (Table 1, Figure 6). There was a significant difference between treatments (Kruskal-Wallis: $p=0.001287$). In the heavy *Sargassum* treatment this portion of the crawl pathway greatly increased the total time.

***Sargassum* Time**

Compared to the crawl control group, hatchlings in the light *Sargassum* treatment group took 426.0% longer to complete the *Sargassum* portion the crawl pathway, and hatchlings in the heavy *Sargassum* treatment group took 295.2% longer (Table 1, Figure 7). The light and heavy *Sargassum* treatment groups took significantly longer than the crawl control, but their times did not significantly differ from each other (Kruskal-Wallis: $p=8.17E-009$. Dunn's: crawl control-heavy $p=0$, crawl control-light $p=0$, light-heavy $p=0.6587$).

Inversions

Heavy *Sargassum* cover was significantly associated with increased instances of hatchlings inverting. Both the crawl control and light *Sargassum* groups had significantly fewer inversions than the heavy *Sargassum* group but did not significantly differ from each other (Kruskal-Wallis: $p=3.88E-05$. Dunn's: crawl control-heavy $p=0$, crawl control-light $p=0.07$, light-heavy $p=0.0182$) (Table 2; Figure 8). Along with the differences in inversions between treatments, both total time and *Sargassum* climb significantly increased as number of

inversions increased (linear regression: light $p=2.94E-06$, $R^2=0.696$, heavy $p=3.67E-02$, $R^2=0.1772$) (Figure 9).

Time Limit

Five hatchlings were not able to crawl the length of the entire pathway within the 30-minute time limit. Each of these hatchlings crawled through the sand portion successfully; however, when they reached the *Sargassum*, they were not able to climb up and over to the end of the pathway.

Blood

Glucose

There was no significant difference between any of the treatment groups (Kruskal-Wallis: $p=0.7754$) (Table 2, Figure 10).

Corticosterone

There was no significant difference between any of the treatment groups (Kruskal-Wallis: $p=0.2508$) (Table 2, Figure 11).

Righting Response

There were no significant differences between righting response in any of the treatments (Kruskal-Wallis: $p=0.4798$) (Table 2, Figure 12).

Hatchling Size

Mean SCL, SCW, FL, body depth, mass, and BCI are summarized in Table 3. In the crawl control treatment longer SCL significantly correlated with

slower total crawl times (Table 7, Figures 13 and 14), all other treatment groups were not significant. SCW, flipper length, and body depth had no significant correlations to crawl times (Table 7, Figures 15-20). Mass was marginally significant with climb time in the heavy *Sargassum* treatment with a larger mass correlating with slower climb times (Table 7, Figures 21 and 22). Body Condition Index was marginally correlated with higher BCI correlating to faster total crawl times and climb times in the crawl control and heavy *Sargassum* treatment groups (Table 7, Figures 23 and 24), all other treatment groups were not significantly correlated.

Table 7: Linear regression statistical output for hatchling body size and crawl times. Significance indicated by * and bold.

SCL-total time			SCW-total time			FL-total time		
CC	p-value	R ²	CC	p-value	R ²	CC	p-value	R ²
Crawl	0.02237*	0.2163	Crawl	0.2296	0.02798	Crawl	0.1506	0.0619
Light	0.9474	-0.05529	Light	0.6962	-0.0464	Light	0.1474	0.06365
Heavy	0.6889	-0.04594	Heavy	0.7966	-0.05156	Heavy	0.4943	-0.02776
SCL-climb time			SCW-climb time			FL-climb time		
Light	0.803	-0.05182	Light	0.6557	-0.04364	Light	0.1636	0.05516
Heavy	0.5542	-0.03467	Heavy	0.5808	-0.03732	Heavy	0.3140	0.003819

Body depth-total time			Mass-total time			BCI-total time		
CC	p-value	R ²	CC	p-value	R ²	CC	p-value	R ²
Crawl	0.3087	0.00509	Crawl	0.1125	0.08584	Crawl	0.0507	0.1512
Light	0.3008	0.007045	Light	0.6011	-0.03921	Light	0.3476	-0.0036
Heavy	0.371	-0.0084	Heavy	0.0921	0.1023	Heavy	0.0287*	0.1966
Body depth-climb time			Mass-climb time			BCI-climb time		
Light	0.5009	-0.0286	Light	0.509	-0.02959	Light	0.4361	-0.0196
Heavy	0.2996	0.00374	Heavy	0.0585	0.1395	Heavy	0.0379*	0.1745

Temperature

Sand

Mean sand temperature was 24.97°C ($\pm 1.46^\circ\text{C}$) with a range of 22.53–28.27 C. There was a significant linear correlation between sand temperature and total time for only the heavy *Sargassum*, with faster crawl times on warmer sand (linear regression for sand time: crawl control $p=0.2168$, $R^2=0.03249$, light $p=0.1359$, $R^2=0.0703$, heavy $p=0.1092$, $R^2=0.08832$. Linear regression for total time: crawl control $p=0.2316$, $R^2=0.02727$, light $p=0.3855$, $R^2=-0.01111$, heavy $p=0.01903$, $R^2=0.2288$) (Figure 25 and 26).

Sargassum

Mean *Sargassum* temperature was 25.47 C (± 1.46) with a range of 22.7 – 28.3C. There were no significant correlations between *Sargassum* temperature and crawl time (linear regression for climb time: light $p=0.9132$, $R^2=-0.05484$, heavy $p=0.07406$, $R^2=0.1202$. Linear regression for *Sargassum* time: light $p=0.6668$, $R^2=-0.04444$, heavy $p=0.2145$, $R^2=0.04781$) (Figure 27 and 28).

Nest

Mean nest temperature can be seen in Table 5. There were no significant linear correlations between nest temperature and crawl time (Table 8). This is true for the overall average nest temperature during the entire incubation period, the maximum temperature recorded, and the highest consecutive three day average temperature (Figure 29-34).

There was no significant difference between nest temperatures and blood plasma corticosterone concentrations (Figures 35-37).

Table 8: Linear regression statistical output for nest temperature correlation to crawl times. There were no significant correlations found.

Mean nest temp- total time			Mean nest temp- climb time			Max nest temp- total time		
	p-value	R ²		p-value	R ²		p-value	R ²
Crawl	0.2911	0.009536	Light	0.6506	-0.04326	Crawl	0.389	-0.01175
Light	0.4494	-0.02162	Heavy	0.2011	0.0385	Light	0.09745	0.09766
Heavy	0.1579	0.05804				Heavy	0.3879	-0.01156

3 day max nest temp -total time			3 day max nest temp-climb time			Max nest temp-climb time		
	p-value	R ²		p-value	R ²		p-value	R ²
Crawl	0.3623	-0.00668	Light	0.2677	0.01598	Light	0.2172	0.03236
Light	0.128	0.07524	Heavy	0.3242	0.001433	Heavy	0.4653	-0.02389
Heavy	0.2795	0.01263						

Green Turtles

Total Time

Hatchlings in the crawl control group required an average of 109 s to crawl the length of the pathway, the light *Sargassum* treatment required an average of 230 s, and the heavy *Sargassum* required an average of 416 s. Hatchlings in the light *Sargassum* treatment had a 110.7% increase in total time when compared to the crawl control group, while those in the heavy *Sargassum* treatment group took 280.9% longer than the crawl control group (Table 1, Figures 3 and 4). The total time to crawl the entire length of the pathway was significantly different the crawl control and both *Sargassum* treatment groups, but the light and heavy *Sargassum* treatments were not significantly different from each other (Kruskal-Wallis: $p=7.39E-09$. Dunn's: crawl control-heavy $p=0$, crawl control-light $p=0$, light-heavy $p=0.1357$), with *Sargassum* cover increasing the total time. This was primarily due to the time it took hatchlings to crawl onto the *Sargassum* pile.

Sand Time

The amount of time to crawl on the sand portion of the crawl pathway (i.e., the first 13 m) was not significantly different between treatment groups (Kruskal-Wallis: $p=0.3655$) (Table 1, Figure 5).

Climb Time

Hatchlings in the heavy *Sargassum* treatment group took 454.2% longer to climb onto the *Sargassum* than hatchlings in the light treatment group (Table 1, Figure 6). There was a significant difference between treatments (Kruskal-Wallis: $p=0.000153$). In the heavy *Sargassum* treatment this portion of the crawl pathway greatly increased the total time.

***Sargassum* Time**

Compared to the crawl control group, hatchlings in the light *Sargassum* treatment group took 264.2% longer to complete the crawl pathway, and hatchlings in the heavy *Sargassum* treatment group took 232.1% longer (Table 1, Figure 7). The light and heavy *Sargassum* treatment groups took significantly longer than the crawl control, but their times did not significantly differ from each other (Kruskal-Wallis: $p=8.17E-09$. Dunn's: crawl control-heavy $p=0$, crawl control-light $p=0$, light-heavy $p=1$).

Inversions

Heavy *Sargassum* cover was significantly associated with increased instances of hatchlings inverting. Both the crawl control and light *Sargassum* groups had significantly fewer inversions than the heavy *Sargassum* group but did not significantly differ from each other ((Kruskal-Wallis: $p=5.92E-06$. Dunn's: crawl control-heavy $p=0$, crawl control-light $p=0.2116$, light-heavy $p=0.0014$). (Table 2; Figure 8). Along with the differences in inversions between treatments, both total time and *Sargassum* climb significantly increased as number of

inversions increased (linear regression: light $p=1.61\text{E-}08$, $R^2=0.8282$, heavy $p=7.23\text{E-}04$, $R^2=0.4499$) (Figure 9).

Time Limit

Two hatchlings were not able to crawl the length of the entire pathway within the 30-minute time limit. Each of these hatchlings crawled through the sand portion successfully; however, when they reached the *Sargassum*, they were not able to climb up and over to the end of the pathway.

Blood

Glucose

There was no significant difference between any of the treatment groups (Kruskal-Wallis: $p=0.1992$) (Table 2, Figure 10).

Corticosterone

There was no significant difference between any of the treatment groups (Kruskal-Wallis: $p=0.7374$) (Table 2, Figure 11)

Righting Response

There were no significant differences between righting response in any of the treatments (Kruskal-Wallis: $p=0.4764$) (Table 2, Figure 12).

Hatchling Size

Mean SCL, SCW, FL, body depth, mass, and BCI are summarized in Table 3. SCL had no significant correlation with crawl times (Table 9, Figures 13

and 14). SCW, flipper length, and body depth, and mass had no significant correlations to crawl times (Table 9, Figures 15-22). Body Condition Index was significantly correlated with higher BCI correlating to faster total crawl times and climb times in the heavy *Sargassum* treatment groups (Table 9, Figures 23 and 24), all other treatment groups were not significant.

Table 9: Linear regression statistical output for hatchling body size and crawl times. Significance indicated by * and bold.

SCL-total time			SCW-total time			FL-total time		
CM	p-value	R ²	CM	p-value	R ²	CM	p-value	R ²
Crawl	0.6418	-0.04259	Crawl	0.5379	-0.03293	Crawl	0.1774	0.0486
Light	0.228	0.02853	Light	0.5082	-0.02949	Light	0.05883	0.1391
Heavy	0.2598	0.01828	Heavy	0.5957	-0.03872	Heavy	0.2487	0.02167
SCL-climb time			SCW-climb time			FL-climb time		
Light	0.9347	-0.05515	Light	0.7719	-0.0505	Light	0.3359	-0.00118
Heavy	0.3413	-0.002346	Heavy	0.7074	-0.0461	Heavy	0.3101	0.004764

Body depth-total time			Mass-total time			BCI-total time		
CM	p-value	R ²	CM	p-value	R ²	CM	p-value	R ²
Crawl	0.6008	-0.03918	Crawl	0.800	-0.05169	Crawl	0.7931	-0.05141
Light	0.6813	-0.04543	Light	0.3065	0.005641	Light	0.3538	-0.00498
Heavy	0.256	0.01943	Heavy	0.7151	-0.04755	Heavy	0.02774*	0.1994
Body depth-climb time			Mass-climb time			BCI-climb time		
Light	0.6852	-0.04569	Light	0.8525	-0.05347	Light	0.9547	-0.05536
Heavy	0.3112	0.004497	Heavy	0.6588	-0.04387	Heavy	0.04295*	0.1646

Temperature

Sand

Mean sand temperature was 25.56°C ($\pm 1.50^\circ\text{C}$) with a range of 22.57–28.67 C. There were no significant linear correlations between sand temperature and crawl times (linear regression for sand time: crawl control $p=0.7909$, $R^2=-$

0.05133, light $p=0.5620$, $R^2=-0.03547$, heavy $p=0.1001$, $R^2=0.09547$. Linear regression for total time: crawl control $p=0.7668$, $R^2=-0.05026$, light $p=0.1004$, $R^2=0.09519$, heavy $p=0.5367$, $R^2=-0.0328$) (Figure 25 and 26).

Sargassum

Mean *Sargassum* temperature was 25.84°C ($\pm 1.70^\circ\text{C}$) with a range of 22.5-28.9°C. There were no significant linear correlations between sand temperature and crawl times (linear regression for climb time: light $p=0.8127$, $R^2=-0.05218$, heavy $p=0.08147$, $R^2=0.1124$. Linear regression for *Sargassum* time: light $p=0.3144$, $R^2=0.003733$, heavy $p=0.2276$, $R^2=0.03267$) (Figure 27 and 28).

Nest

Average nest temperatures can be seen in Table 5. Nests that had higher overall average incubation temperatures had significantly slower total times and *Sargassum* climb time in both the light and heavy *Sargassum* treatment groups (Table 10, Figure 29 and 30). There were no other significant linear correlations between nest temperature and crawl times (Table 10, Figures 31-34).

There was a significant difference in blood plasma corticosterone concentrations in both the lowest and highest nest incubation temperatures when compared to the middle nest incubation temperatures (Figures 35-37).

Table 10: Linear regression statistical output for nest temperature correlation to crawl times. Significance indicated by bold and *.

Mean nest temp-total time			Mean nest temp-climb time			Max nest temp-total time		
	p-value	R ²		p-value	R ²		p-value	R ²
Crawl	0.33	0.000128	Light	0.1141	0.08467	Crawl	0.2905	0.009688
Light	0.02157 *	0.2191	Heavy	0.03446 *	0.1822	Light	0.2371	0.02544
Heavy	0.02297 *	0.2142				Heavy	0.1651	0.05443

Max nest temp- climb time			3 day max nest temp-total time			3 day max nest temp-climb time		
	p-value	R ²		p-value	R ²		p-value	R ²
Light	0.1656	0.0542	Crawl	0.2869	0.01064	Light	0.5493	-0.03416
Heavy	0.2313	0.02739	Light	0.8822	-0.05423	Heavy	0.1937	0.0415
			Heavy	0.1355	0.07055			

Between Species Comparison

Total Time

All species showed an increase in the total time it took hatchlings to crawl the entire pathway with the addition of *Sargassum*. In general leatherbacks and loggerheads had similar total times, with leatherbacks being slightly slower in the crawl control treatment. Green turtles were significantly faster than the other two species in every treatment group (Figure 3 and 4).

Sand Time

There were no differences in sand time between treatments for any species. Sand time was significantly different between all species with leatherbacks having the slowest crawl time followed by loggerheads, then green turtles (Figure 5).

Climb Time

All species had an increase in climb time as the *Sargassum* height increased. Also, all species had climb times in the heavy *Sargassum* treatment that greatly increased the total time (Figure 3). In the light *Sargassum* treatment leatherbacks and green turtles had similar climb times while loggerheads were significantly slower. In the heavy *Sargassum* treatment leatherbacks and

loggerheads had similar climb times and green turtles were significantly faster (Figure 6).

***Sargassum* Time**

For all three species, crawl times did not significantly differ between the light and heavy *Sargassum* treatments. Leatherbacks and loggerheads were significantly slower than green turtles, but not significantly different from each other (Figure 7).

Inversions

The number of inversions significantly increased in the heavy *Sargassum* treatment in all species (Figure 8). Also, all species had a significant increase in both total time and *Sargassum* climb time as the number of inversions increased (Figure 9). The number of inversions between species was not significantly different in any treatment group.

Blood

Glucose

Blood glucose concentrations remained the same between treatment groups within each species, with the exception of the leatherback in the no crawl control. There was no significant difference in glucose levels between species in the no crawl control. However, in all of the crawling trials loggerheads and green turtles had significantly higher glucose levels than leatherbacks but were not significantly different from each other (Figure 10).

Corticosterone

Blood plasma corticosterone concentrations remained the same between treatment groups within each species (Figure 11). Leatherbacks had significantly different lower CORT for all treatment groups than loggerhead or green turtles, but loggerheads and green turtles were not significantly different than each other.

Righting Response

No species showed a difference in righting response between treatment groups within the same species, though there were differences between species (Figure 12). Leatherbacks were significantly slower than loggerheads and green turtles in all treatment groups. In the no crawl control as well as the crawl control green turtles were significantly faster than loggerheads, however in the light and heavy *Sargassum* treatment green turtles and loggerheads were not significantly different.

Nest Temperature

Green turtles were the only species that had significant correlations between higher nest temperature and slower crawling times. Leatherback nests were significantly cooler than loggerhead and green turtle nests in all categories (Table 5). Loggerhead and green turtle nests had very similar temperatures.

Discussion

Overall Findings

The addition of more *Sargassum* in the crawl pathway increased the amount of time hatchlings of all three species spent crawling down the pathway on the beach (leatherback: 34.7% increase in light treatment and 233.4% increase in heavy; loggerhead: 121.8% increase in light treatment and 284.7% increase in heavy; green turtle: 110.7% increase in light treatment and 281.0% increase in heavy). The majority of the extra time spent crawling in the pathway occurred while hatchlings were climbing from the sand to the top of the *Sargassum* pile (Figure 3). Once on top of the pile, crawling over the *Sargassum* itself also added extra time when compared to crawling on only sand. However, there was not a significant difference in *Sargassum* crawl time between the light and heavy *Sargassum* treatment groups, indicating that the height of *Sargassum* had no impact once hatchlings climbed to the top of the pile. Some hatchlings were also not able to crawl up and over the *Sargassum* and reached the time limit; it can be assumed that these hatchlings would have never made it past the *Sargassum*. The addition of *Sargassum* did not have an impact on hatchlings physical abilities or physiology in any of the species for the factors examined in this study. Righting response, glucose concentrations, and corticosterone concentrations were not

different between crawling treatment groups, so the height of *Sargassum* did not have an impact.

Increased time spent on beach

The largest impact *Sargassum* had on hatchlings crawling to the water was increasing the amount of time they spend crawling on the beach from the nest to the water, which would likely increase their risk of predation (Erb and Wyneken, 2019; Tomillo et al., 2010). This finding was similar to an earlier study that found that crawling over seaweed on the beach decreased loggerhead hatchling crawl speed (Triessnig et al., 2012). Another study found that a small number of loggerhead and green turtle hatchlings struggled to crawl through *Sargassum* while crawling to the water (Rodríguez-Martínez et al., 2021). The current study only examined *Sargassum* heights up to 19 cm, so in areas in which *Sargassum* accumulation is even higher it can be expected that the amount of time hatchlings will spend to navigate this obstacle will increase even further with the possibility of hatchlings not being able to climb over the pile (Schiariti and Salmon, 2022). There were multiple hatchlings in this study that were not able to climb up the pile within the time limit, so as the height of *Sargassum* increases, the number of hatchlings failing to make it over the *Sargassum* will also likely increase. For this study, hatchling only had to crawl over one pile of *Sargassum*; however, on some beaches there will be multiple bands of *Sargassum* over which hatchlings have to climb. Climbing up the *Sargassum* created the largest increase in time spent on the beach, so climbing over multiple piles will further increase time spent crawling down the beach to the water. It was also found that crawling over

Sargassum did increase time spent crawling when compared to bare sand regardless of *Sargassum* height, so even very light but widespread beach coverage would increase amount of time spent on the beach.

During the heavy *Sargassum* trials, the number of times hatchlings inverted also significantly increased, with five hatchlings inverting over 20 times while trying to navigate up the *Sargassum*. This increase in inversions is significantly correlated with an increase in total time as well as *Sargassum* climb time. Spending time upside down on the beach makes hatchlings more vulnerable to predation and if they fail to right themselves, this can lead to mortality (Tomillo et al., 2010). Hatchlings are trying to move as quickly as possible down the beach to try to avoid predators while traveling to the water (Burger and Gochfeld, 2014; Spencer and Janzen, 2011), so any added time spent on the beach when encountering *Sargassum* increases the period of time they are exposed to potential predators.

Physiological response to crawling over *Sargassum*

Glucose concentrations post-crawl did not significantly differ by treatment group. Only leatherback hatchlings in the no crawl control had significantly higher glucose concentrations when compared to the other groups within the species. This shows that crawling down the beach does have an impact on blood analytes, especially in leatherbacks, but the addition of *Sargassum* did not have a further impact. A reduction in glucose levels can have an impact on hatchling locomotion since it acts as a fuel source (Hamann et al., 2007); however this does not appear to be a large concern while crawling over a pile of *Sargassum* on the

way to the water since a drastic drop in glucose was not seen. Studies observing hatchlings crawling up to 500 meters and swimming for 2 hours did not see significantly lower glucose levels when compared to hatchlings that did not crawl or swim (Hamann et al., 2007; Pankaew and Milton, 2018; Pereira et al., 2012), so it makes sense that in this study no difference was observed since hatchlings were only crawling 15 meters with a 30 or 45 minute time limit. If hatchlings were to face higher piles of *Sargassum* that they struggled to climb over for an extended period of time, then a difference in blood glucose may be observed and may have an impact on energy reserves required to swim during the frenzy period. Glucose levels observed in this study for all species were similar to previously reported values (Fleming et al., 2020; Hamann et al., 2007; Pereira et al., 2012; Perrault et al., 2022).

As with glucose values, Corticosterone concentrations post-crawl did not significantly change between treatment groups, showing that crawling over *Sargassum* on the beach does not have an impact on this stress hormone. Overall recorded CORT concentrations were high, which likely reflects the high intensity of activity required to emerge from the nest and crawl down the beach to the ocean (Hamann et al., 2007). Measured CORT for loggerhead and green turtle hatchlings are in range with other studies findings, though loggerhead hatchlings were on the higher end (Hamann et al., 2007; Henaghan, 2018; Pereira et al., 2012; Usategui-Martín et al., 2021). Corticosterone concentrations in leatherback hatchlings have not yet been studied, however CORT in hatchlings from this

study were similar to CORT concentrations found in nesting leatherback females (Rostal et al., 2001).

Physical response to crawling over *Sargassum*

In order to measure a physical response in the hatchlings, a righting response was used as a proxy for physical fitness with longer righting response times correlating with lower physical abilities (Delmas et al., 2007; Dial, 1987). Conducting the righting response in water mimics hatchlings becoming overturned once reaching the ocean during their frenzy (Fleming et al., 2020). There was no significant difference between righting response times within species for the various treatment groups, so crawling over various amounts of *Sargassum* does not appear to have an impact on the physical ability of hatchlings. A lack of difference in righting response times suggests that crawling over *Sargassum* does not significantly hinder their ability to flip themselves over if turned upside down by a wave after encountering *Sargassum* on the beach. However, righting response also played a role while hatchlings were climbing up the *Sargassum* pile. In the trials with higher *Sargassum* piles, the number of times hatchlings inverted increased, so hatchlings had to right themselves to continue their crawl. No human interaction occurred when hatchlings flipped upside down, as they were left to right themselves on their own. There was no significant correlation between number of inversions and righting response in the water. This most likely is because righting on land uses different muscles and body parts than swimming, so a righting response in water is more similar to swimming than self-righting on land (Booth et al., 2013).

Species differentials

All species followed similar patterns throughout the different treatment groups in terms of crawl times with an increase in total time as *Sargassum* increased. Overall green turtles crawled much faster than leatherback and loggerhead hatchlings. This suggests that with more *Sargassum*, leatherbacks and loggerheads would be more vulnerable to predation than green turtles when navigating *Sargassum* since they would spend more time on the beach. Overall, green turtles' ability to navigate down the beach and over *Sargassum* faster than the other two species may be due to the fact that they are emerging from nests higher up on the beach closer to the dunes. Perhaps they are more suited to climb over vegetation since they are more likely to encounter beach plants up in the dunes than the other two species. Green turtles also have a larger aerobic scope which may help them to move more quickly (Lutcave and Lutz, 1986).

Leatherbacks may have had the slowest times because their body shape and flipper are adaptations for long distance swimming and deep diving compared to other sea turtle species. Because of the need to perform these activities while in the ocean they may not be able to navigate on land and over vegetation as easily as loggerhead and green turtle hatchlings. They also lack claws on their flippers which may help loggerhead and green turtle hatchlings grip onto the *Sargassum* more easily.

Based on observations during the study, *Sargassum* was washed up on the beach from March until September 2021 with a peak from May to July. This seasonal change in *Sargassum* makes leatherbacks and loggerheads more

susceptible to encountering *Sargassum* based on when these species are hatching in south Florida. Green turtles hatch out later in the season after the main peak of *Sargassum* accumulation has occurred. So, while green turtles were the fastest at navigating through the *Sargassum* they are the species that this macroalgae will affect the least. However, on beaches in Florida some green turtle hatch outs may begin in July, *Sargassum* may have a greater impact than areas with later season green turtle nests.

Impact of environmental factors

As reptiles, sea turtle metabolism and performance are greatly impacted by environmental temperatures, sand temperature. Moving more quickly over warmer sand has been documented in olive ridley sea turtle hatchlings (Burger and Gochfeld, 2014). While sand temperature only had a significant effect on loggerhead hatchlings in some treatment groups, all species did show faster crawl times when the sand was warmer. Higher *Sargassum* temperature also showed the same general trend of faster crawl times, however it was not significant, which may have occurred because there was a narrow range of temperatures as well as a low sample size. Sand temperatures were all within 6°C of each other and *Sargassum* temperatures were within 8°C of each other. The sand temperatures may have not been a wide enough spread of temperatures to be able to see a Q_{10} effect on locomotion. While the *Sargassum* temperature range was larger, it may have been that the temperatures were warm enough that a further change in performance was not observed or that air and sand temperature made more of an

impact since hatchlings typically were in contact with *Sargassum* for the shortest amount of time in the crawl pathway.

Nest incubation temperature also had a significant effect on crawl times only in green turtle hatchlings, with warmer nests producing hatchlings that crawled more slowly through the pathway. A decreased locomotive performance from warmer nests has been documented in previous studies in sea turtle hatchlings (Booth, 2017; Fleming et al., 2020; Ischer et al., 2009; Seaman, 2020). But while green turtle hatchlings that emerge after experiencing higher incubation temperatures will take more time to navigate *Sargassum* on the beach and thus more time spent crawling to the water, even the slower green turtles crawl more quickly than the loggerhead or leatherback hatchlings.

Overall potential impact

The most important factor regarding the addition of *Sargassum* to sea turtles on nesting beaches is the added time spent on the beach while crawling to the water. There does not appear to be a large physical or physiological effect in hatchlings with increasing *Sargassum* abundance, so exposure to potential predators for longer periods of time will have the largest impact. However, when *Sargassum* accumulates to a height in which hatchlings can no longer crawl over it (~30 cm; Schiariti and Salmon, 2022), there may be increased physical and physiological impacts. This may be caused by hatchlings spending longer amounts of time and energy trying to climb over the obstacles. This study did not account for hatchlings trying to navigate around the *Sargassum* since they were in an enclosed pathway, so when *Sargassum* is too high for hatchlings to crawl over,

they may expend time and energy trying to circumvent the *Sargassum*. However, when *Sargassum* washes up it can cover kilometers of the beach and hatchlings' ability to avoid this is unlikely

The *Sargassum* phenomenon continues to increase and will have a larger impact on sea turtles as more mats wash onto the beach. Along with the impact on hatchlings observed in this study, *Sargassum* has been observed having a negative impact on the amount of available nesting beach as well as altering incubating nest temperatures (Maurer et al., 2022, 2021, 2019). While the complete removal of *Sargassum* from the beach is not necessarily the best option for the ecosystem (Williams et al., 2008; Williams and Feagin, 2010), the amount of *Sargassum* accumulating does have an impact on sea turtles and needs to be taken into consideration when creating management plans. Possible considerations are to remove *Sargassum* when heights reach over 30 cm. Large amounts of *Sargassum* would reduce the number of hatchlings that make it to the water and should be accounted for in end of year hatchling estimates.

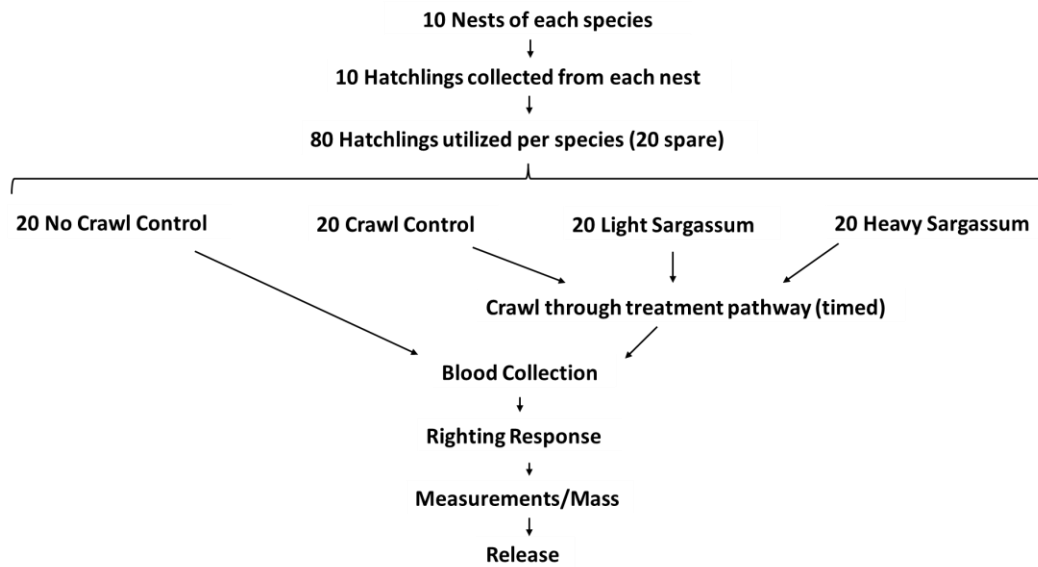


Figure 1: Flow chart of the methods.

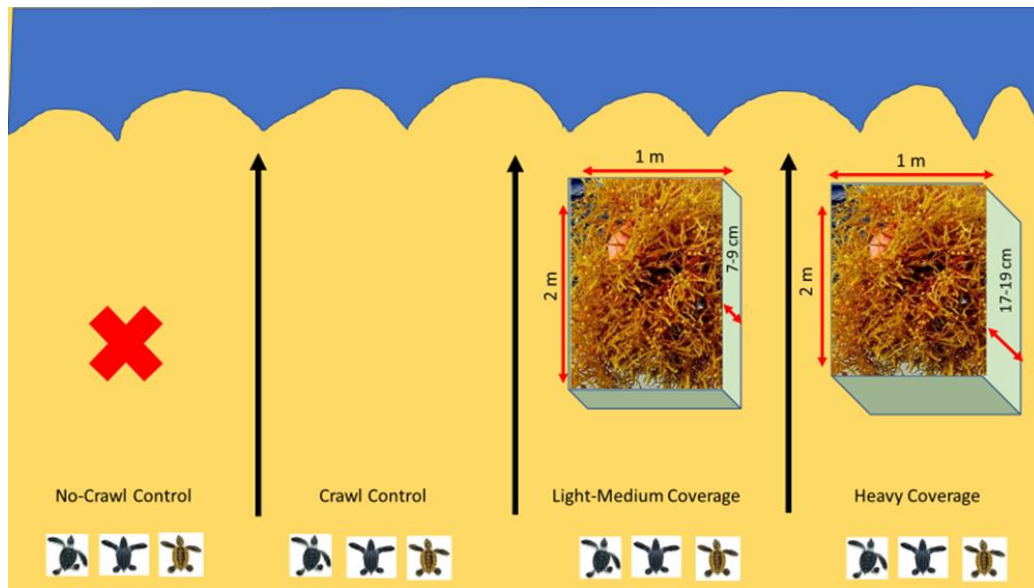


Figure 2: Schematic of the end of the crawl pathway with various amount of *Sargassum*.

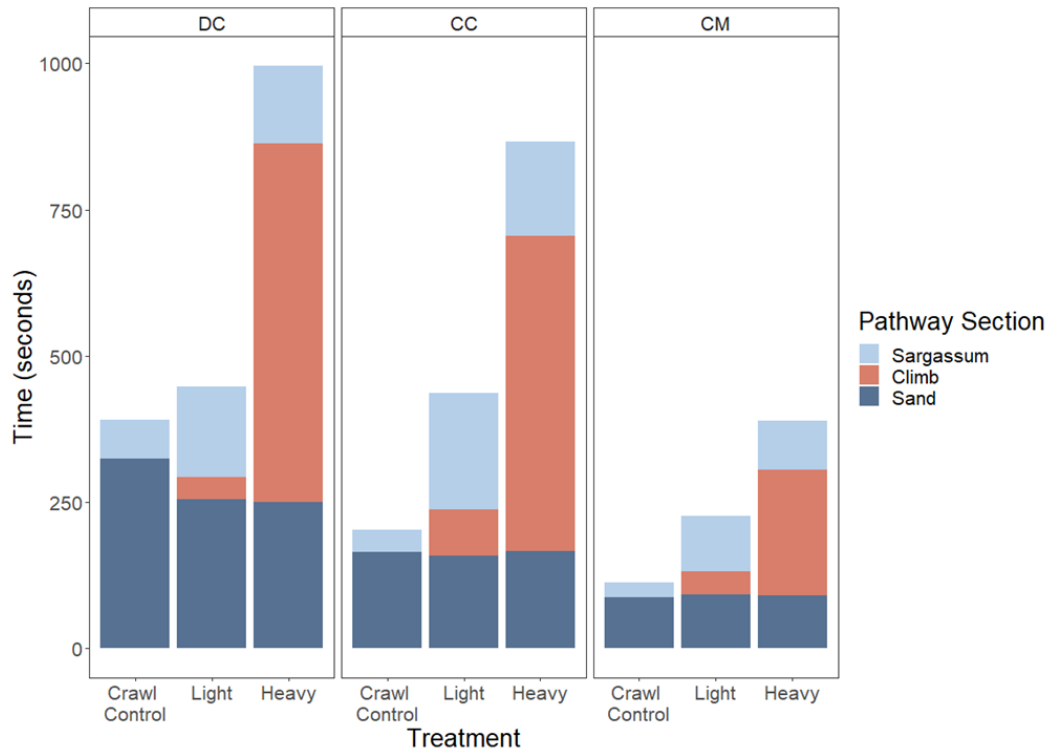


Figure 3: Breakdown of the average total time into pathway sections for each species in each treatment group. Sand: time to crawl the first 13 meters on sand only. Climb: time to climb up to the top of the *Sargassum* pile. *Sargassum*: time to crawl the last 2 meters through various amounts of *Sargassum*.

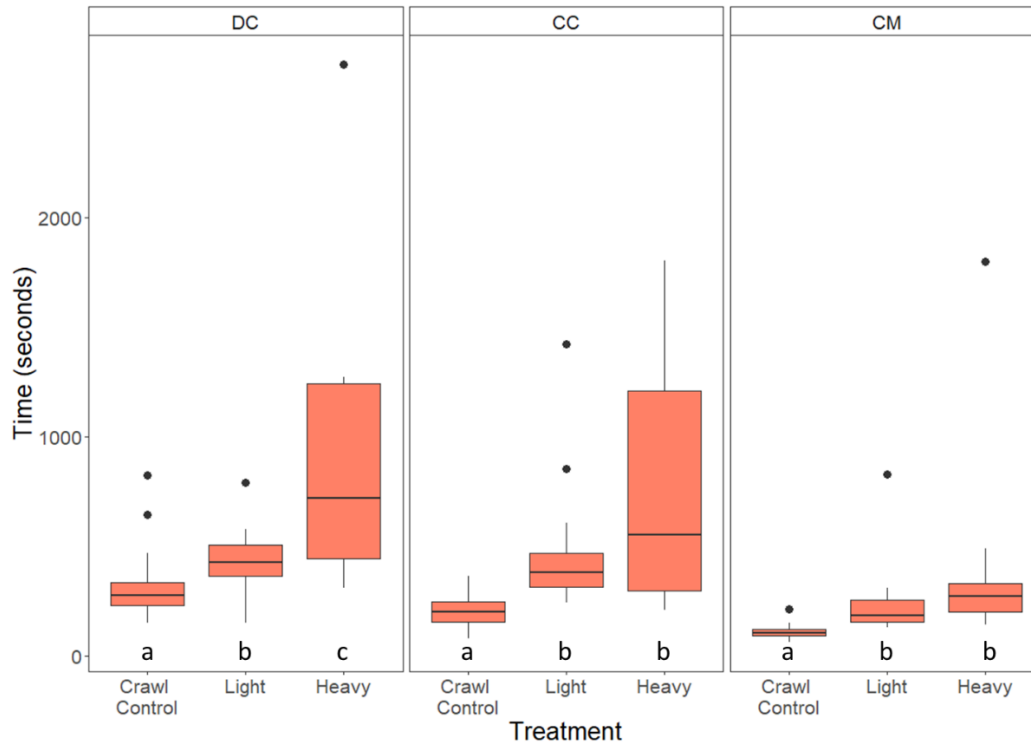


Figure 4: Total time for each species to crawl in each of the different treatment groups. The bottom line of the box represents the 25th percentile, the line in the middle of the box is the median, and the top line of the box is the 75th percentile. Error bars show the 10th and 90th percentile and outliers are indicated as dots. Significance shown using letters, boxes with different letters have significantly different medians. Significance only identified within each species, not between species.

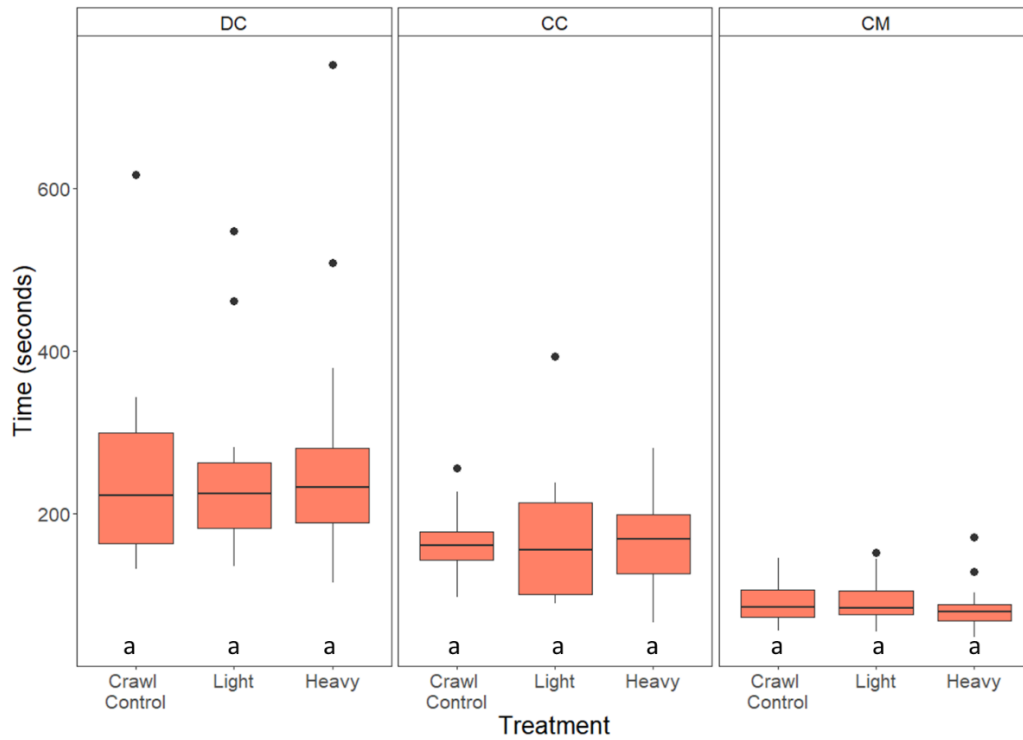


Figure 5: Sand time for each species to crawl in each of the different treatment groups. The bottom line of the box represents the 25th percentile, the line in the middle of the box is the median, and the top line of the box is the 75th percentile. Error bars show the 10th and 90th percentile and outliers are indicated as dots. Significance shown using letters, boxes with different letters have significantly different medians. Significance only identified within each species, not between species.

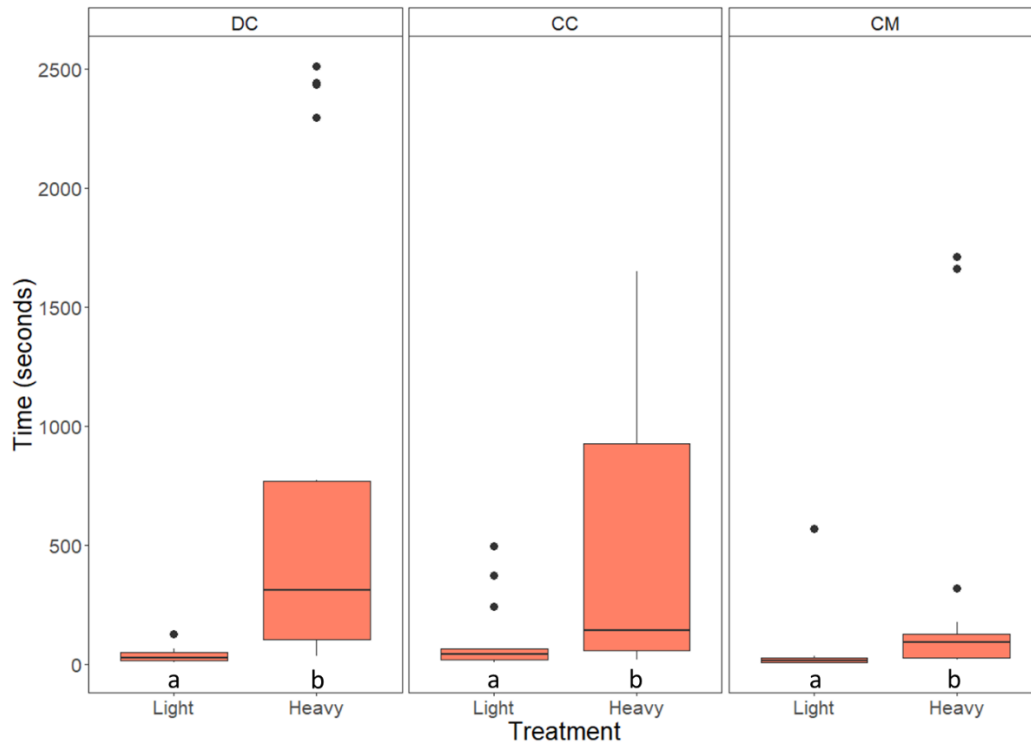


Figure 6: *Sargassum* climb time for each species to crawl in each of the different treatment groups. The bottom line of the box represents the 25th percentile, the line in the middle of the box is the median, and the top line of the box is the 75th percentile. Error bars show the 10th and 90th percentile and outliers are indicated as dots. Significance shown using letters, boxes with different letters have significantly different medians. Significance only identified within each species, not between species.

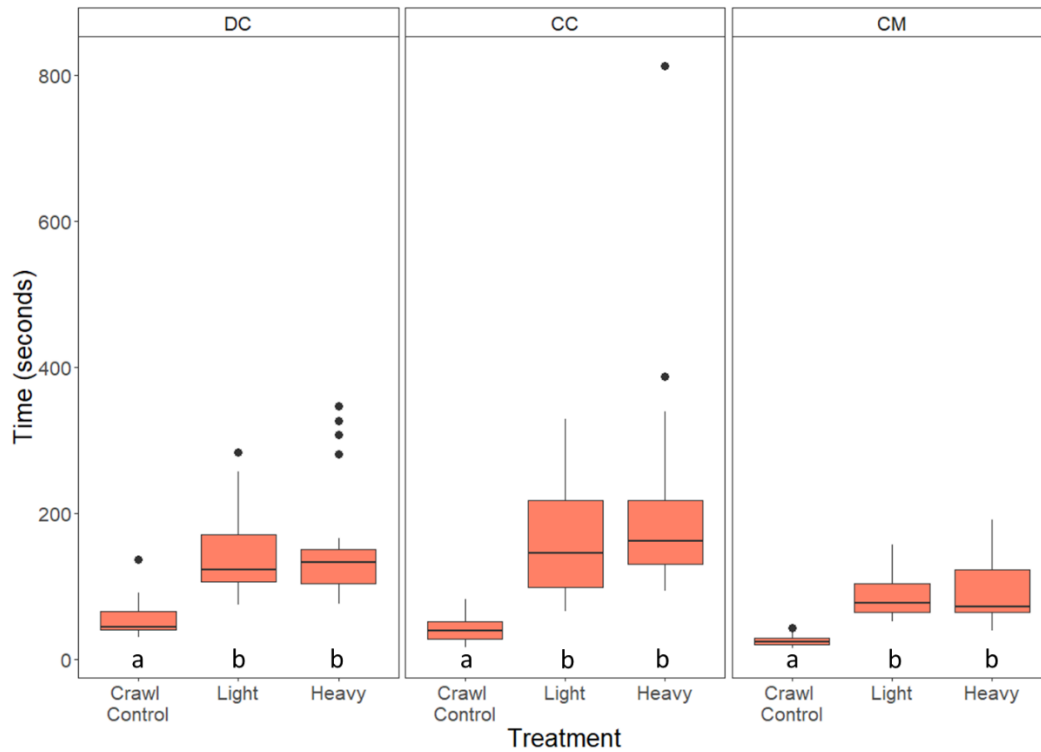


Figure 7: *Sargassum* time for each species to crawl in each of the different treatment groups. The bottom line of the box represents the 25th percentile, the line in the middle of the box is the median, and the top line of the box is the 75th percentile. Error bars show the 10th and 90th percentile and outliers are indicated as dots. Significance shown using letters, boxes with different letters have significantly different medians. Significance only identified within each species, not between species.

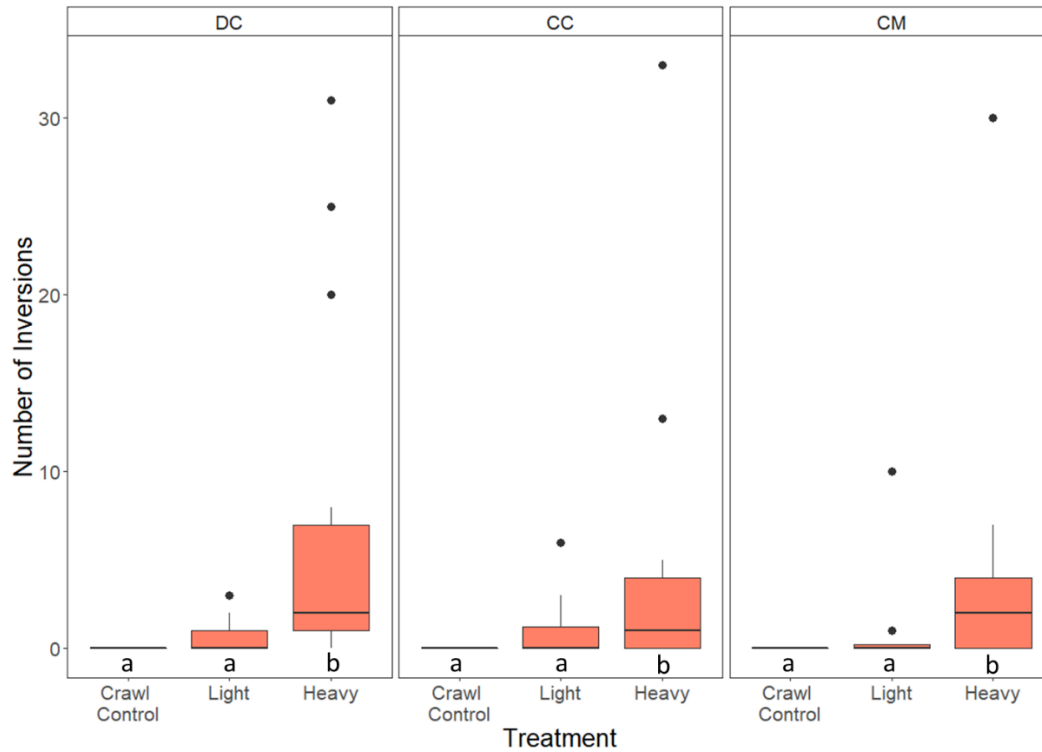


Figure 8: Number of inversions while crawling in the pathway for each species in each of the different treatment groups. The bottom line of the box represents the 25th percentile, the line in the middle of the box is the median, and the top line of the box is the 75th percentile. Error bars show the 10th and 90th percentile and outliers are indicated as dots. Significance shown using letters, boxes with different letters have significantly different medians. Significance only identified within each species, not between species.

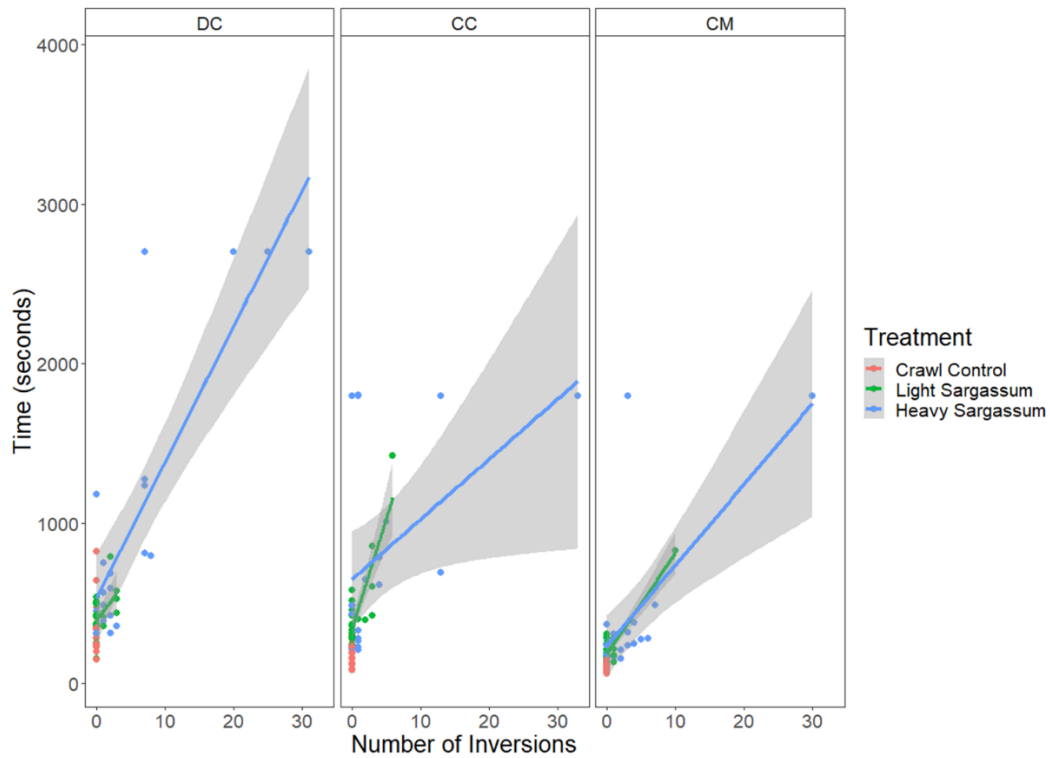


Figure 9: Correlation between number of times hatchlings inverted in the crawl pathway and total time to crawl the length of the entire pathway. Line shows the linear model, grey area is the 95% confidence interval.

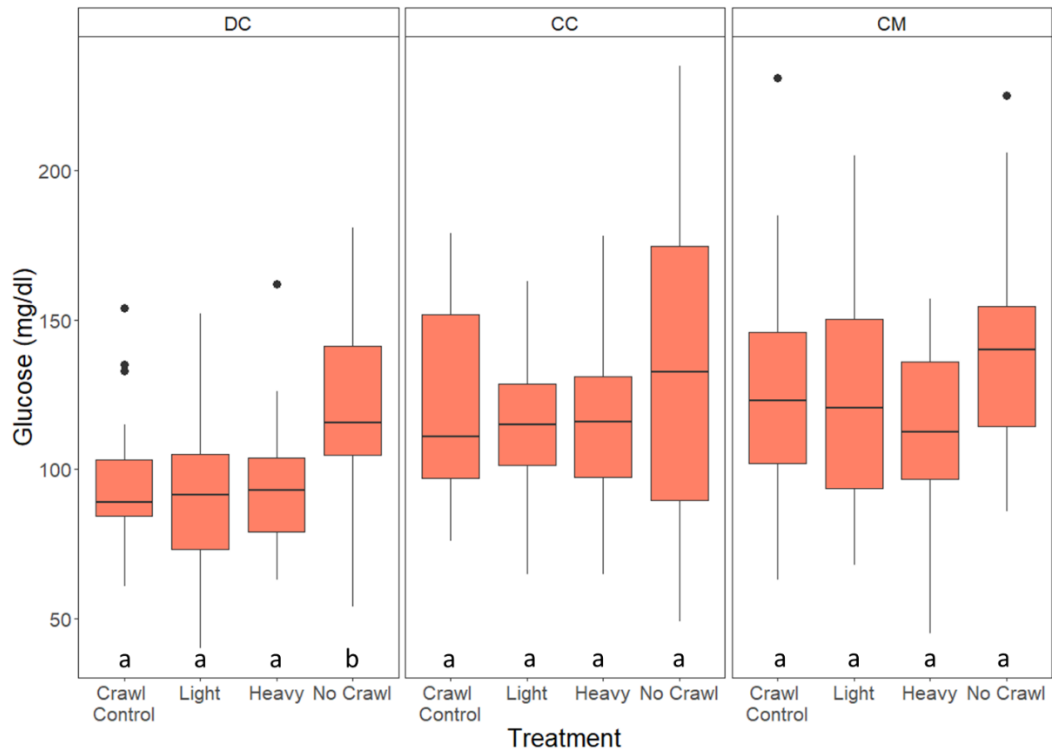


Figure 10: Blood glucose levels post crawl for each species in each of the different treatment groups. The bottom line of the box represents the 25th percentile, the line in the middle of the box is the median, and the top line of the box is the 75th percentile. Error bars show the 10th and 90th percentile and outliers are indicated as dots. Significance shown using letters, boxes with different letters have significantly different medians. Significance only identified within each species, not between species.

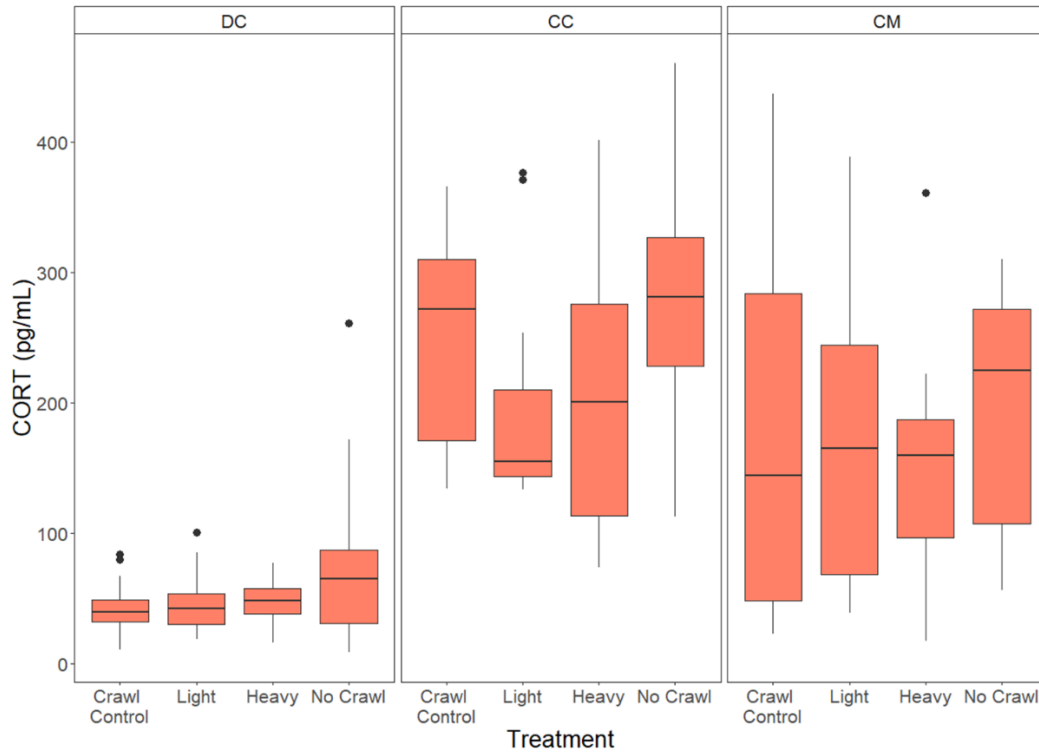


Figure 11: Blood plasma corticosterone levels post crawl for each species in each of the different treatment groups. The bottom line of the box represents the 25th percentile, the line in the middle of the box is the median, and the top line of the box is the 75th percentile. Error bars show the 10th and 90th percentile and outliers are indicated as dots. Significance shown using letters, boxes with different letters have significantly different medians. There was no significant differences between treatment groups within species.

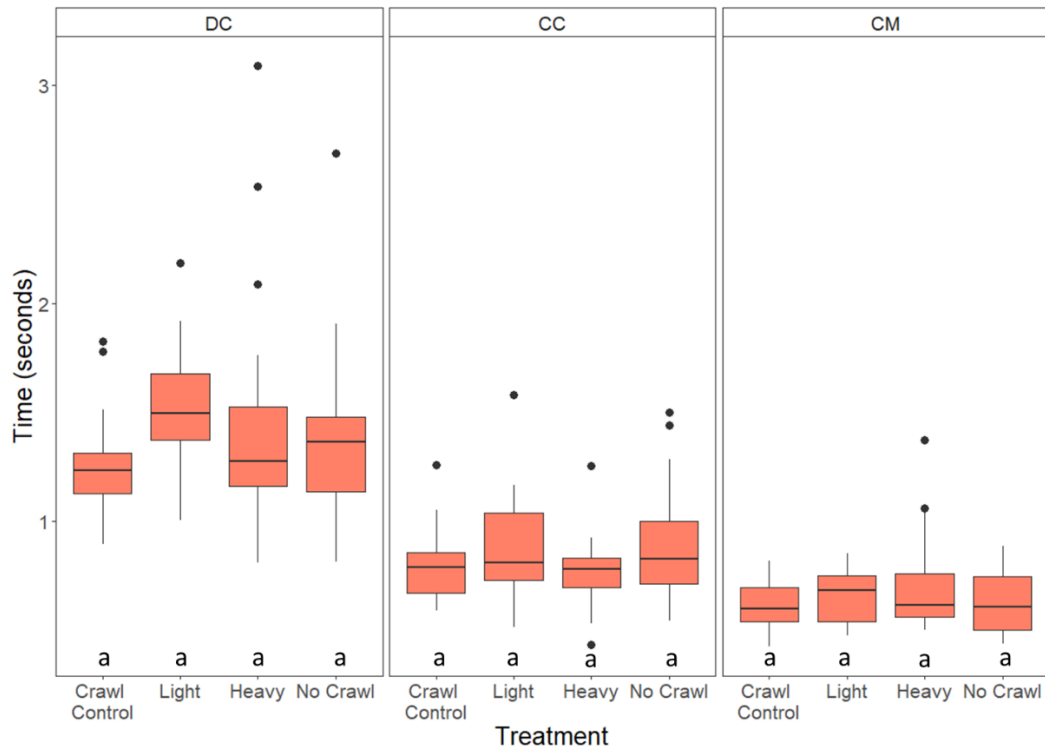


Figure 12: Righting response time post crawl for each species in each of the different treatment groups. The bottom line of the box represents the 25th percentile, the line in the middle of the box is the median, and the top line of the box is the 75th percentile. Error bars show the 10th and 90th percentile and outliers are indicated as dots. Significance shown using letters, boxes with different letters have significantly different medians. Significance only identified within each species, not between species.

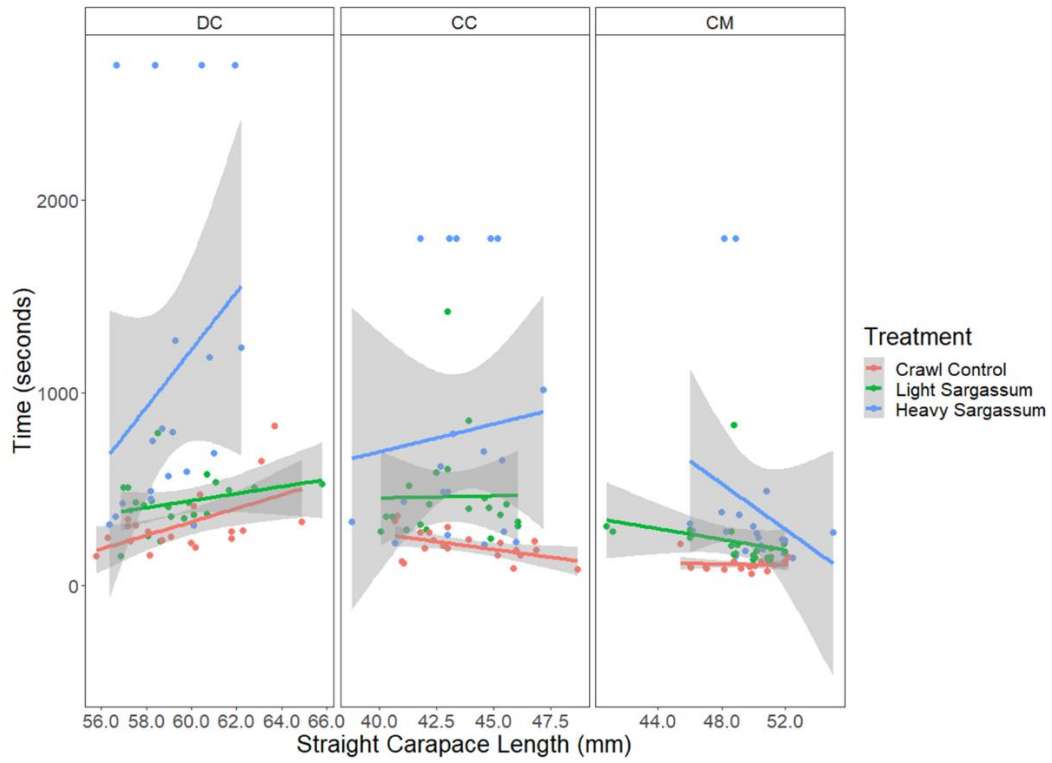


Figure 13: Correlation between straight carapace length (SCL) and total time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

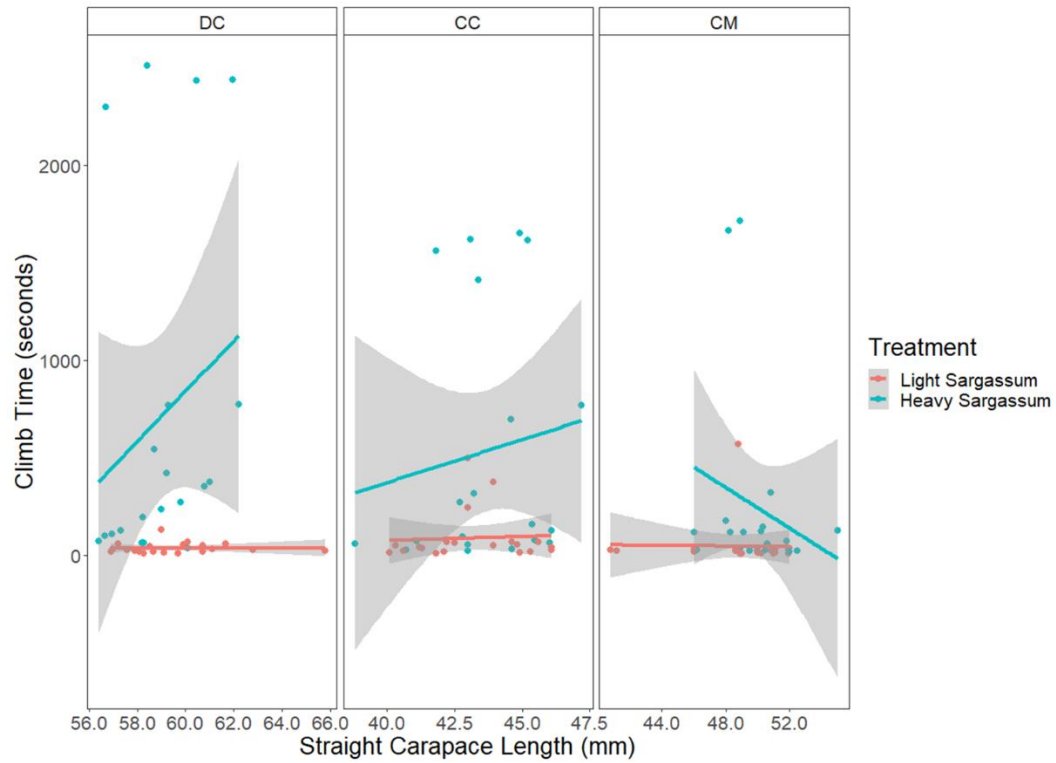


Figure 14: Correlation between straight carapace length (SCL) and time to climb up *Sargassum* pile for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

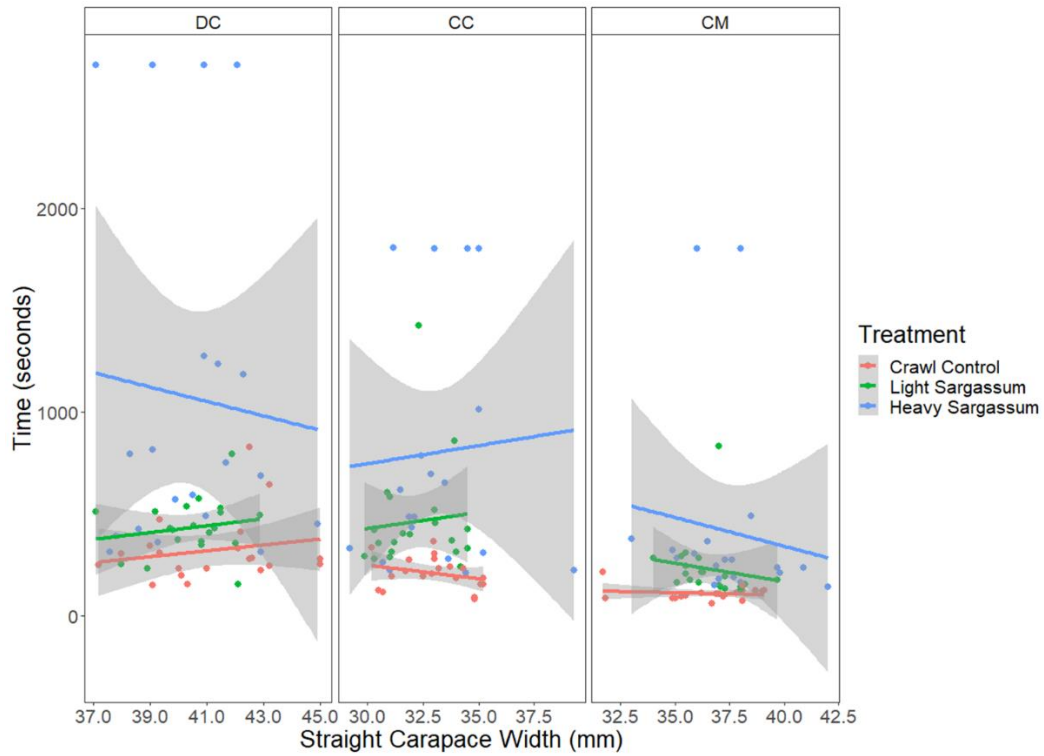


Figure 15: Correlation between straight carapace width (SCW) and total time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

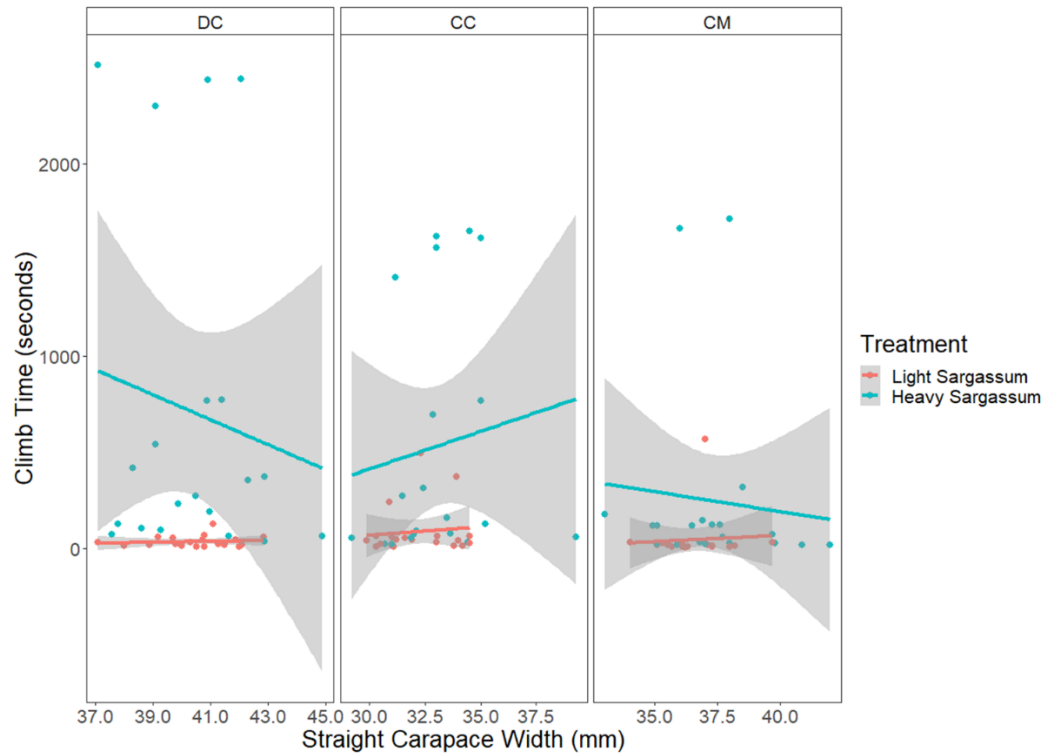


Figure 16: Correlation between straight carapace width (SCW) and time to climb up *Sargassum* pile for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

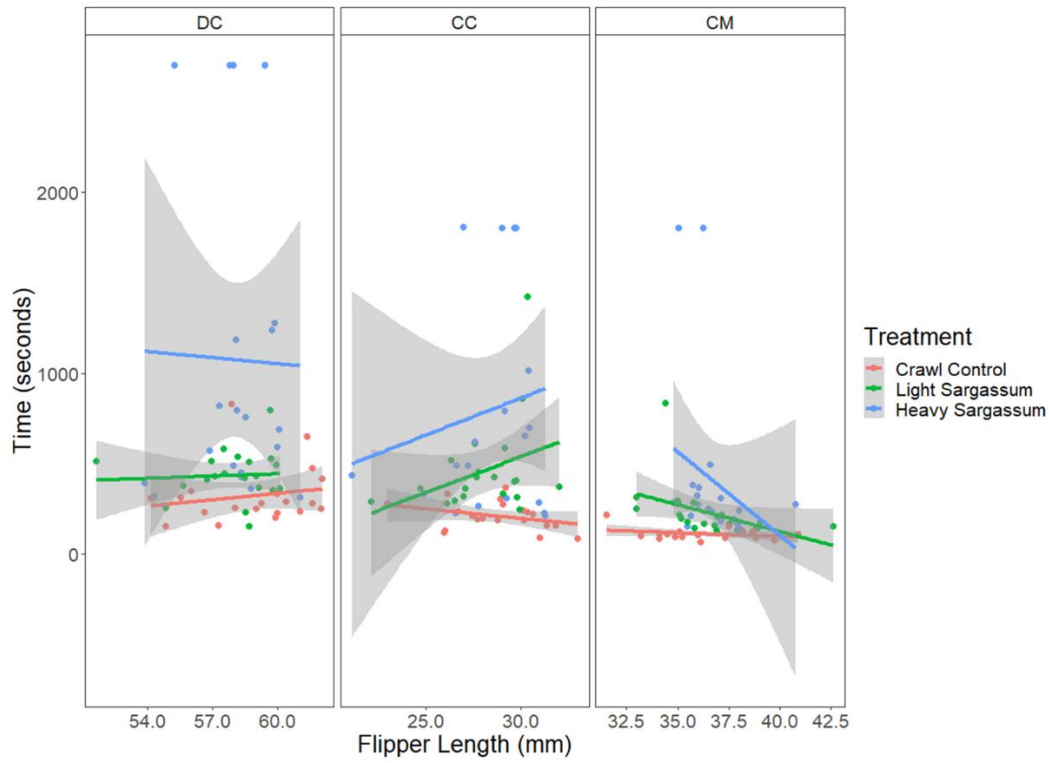


Figure 17: Correlation between flipper length (FL) and total time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

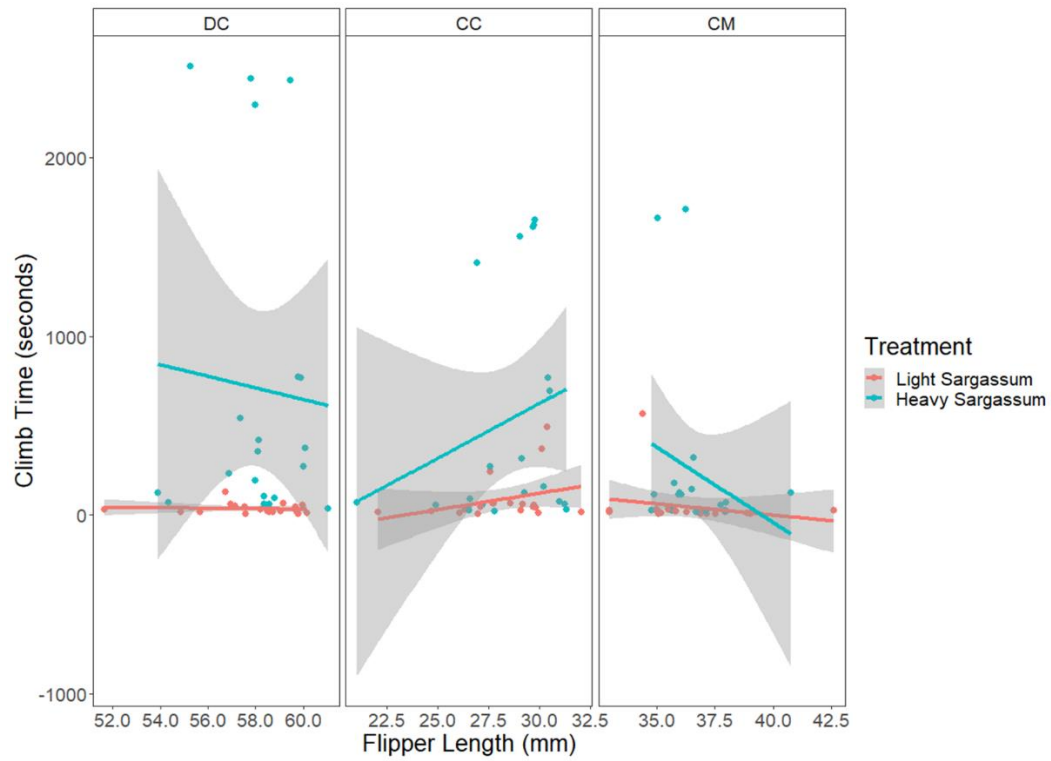


Figure 18: Correlation between flipper length (FL) and time to climb up *Sargassum* pile for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

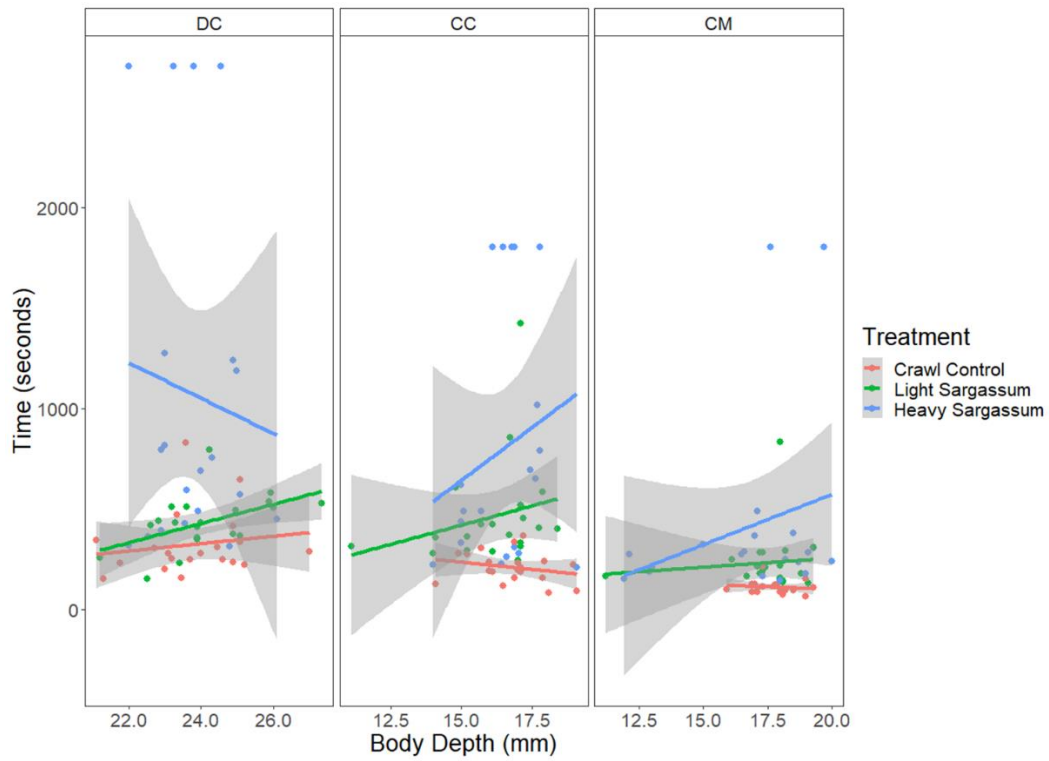


Figure 19: Correlation between body depth and total time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

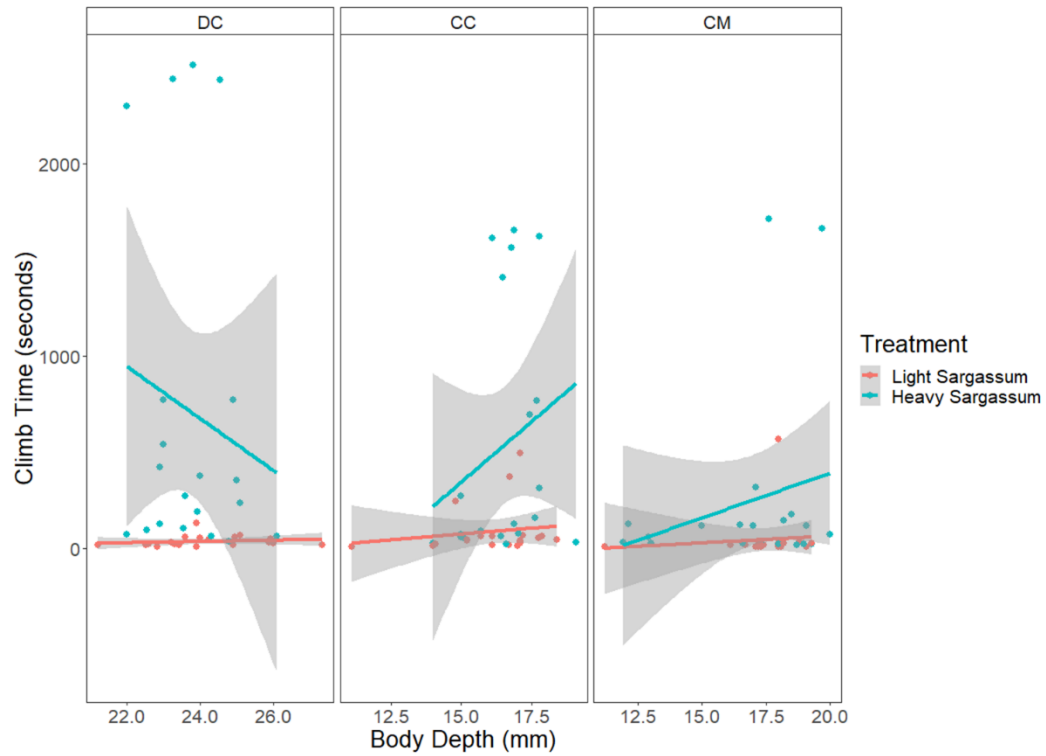


Figure 20: Correlation between body depth and time to climb up *Sargassum* pile for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

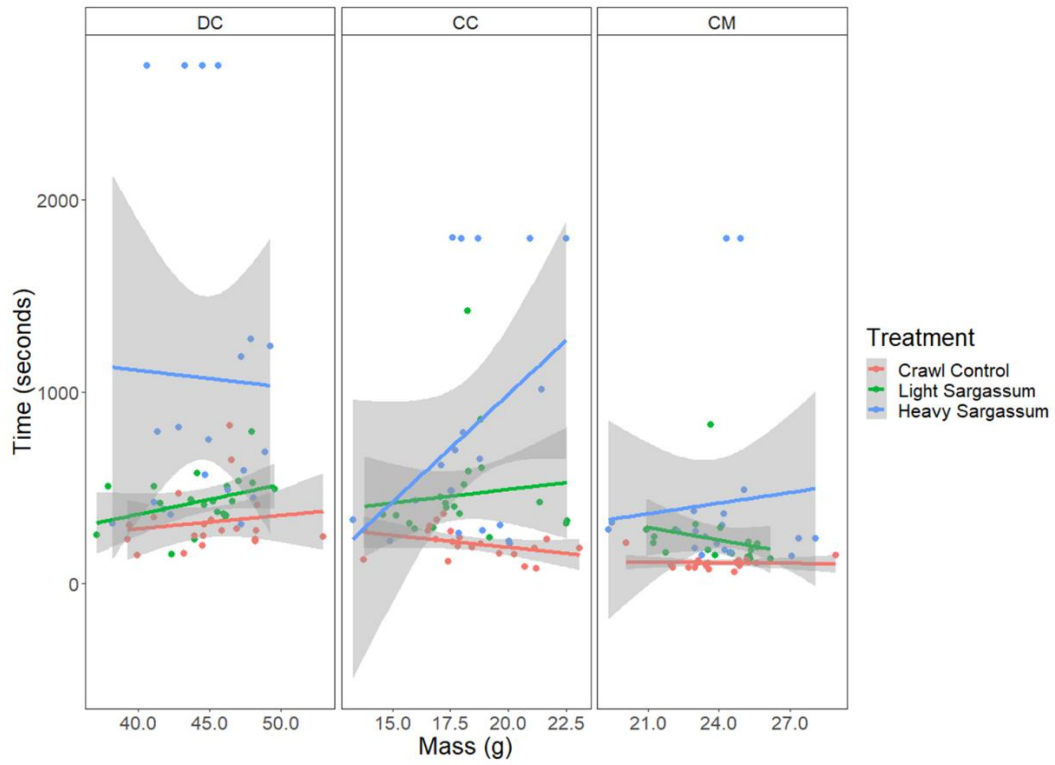


Figure 21: Correlation between mass and total time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

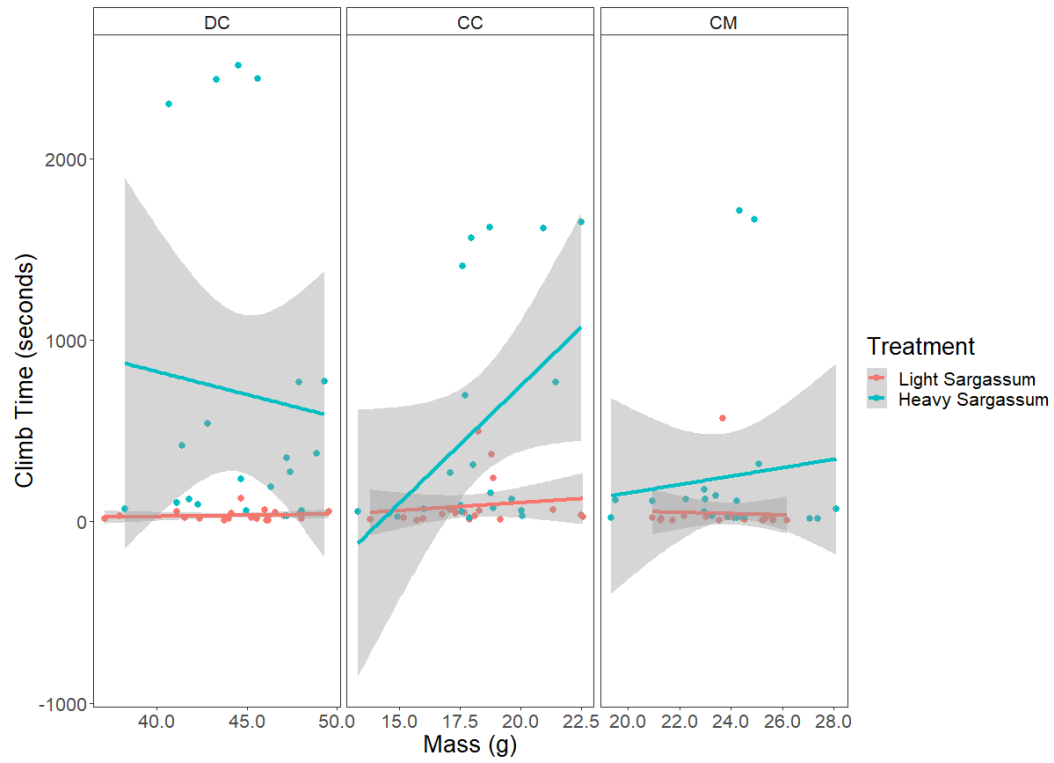


Figure 22: Correlation between mass and time to climb up *Sargassum* pile for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

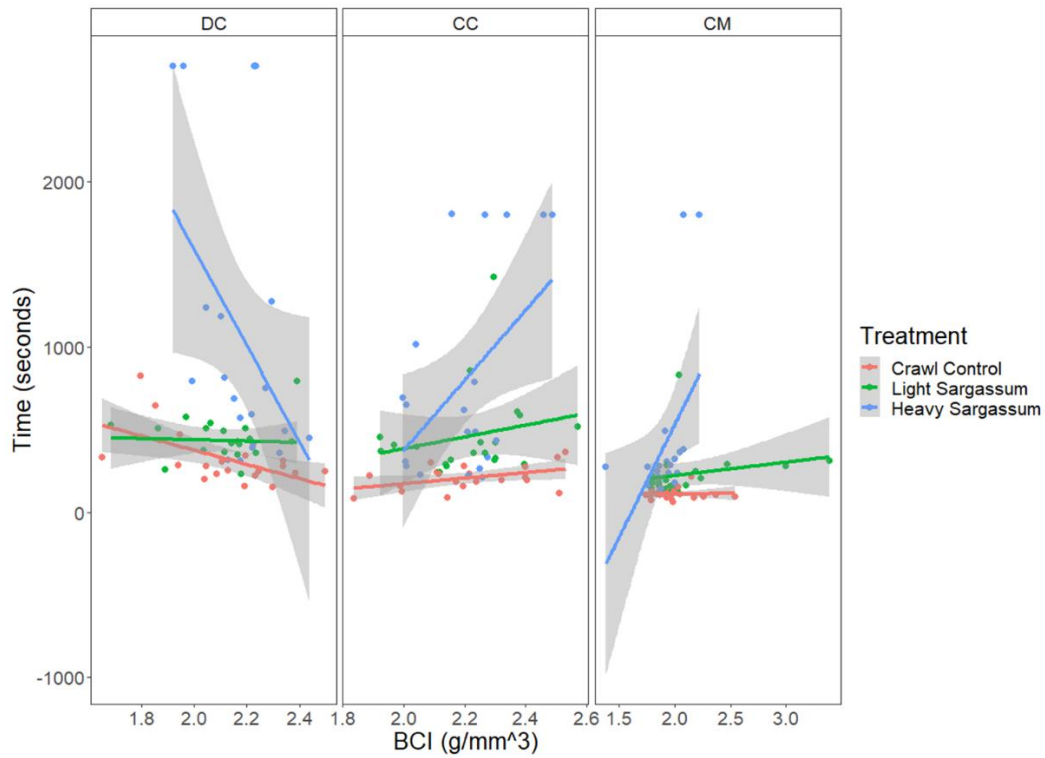


Figure 23: Correlation between body condition index (BCI) and total time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

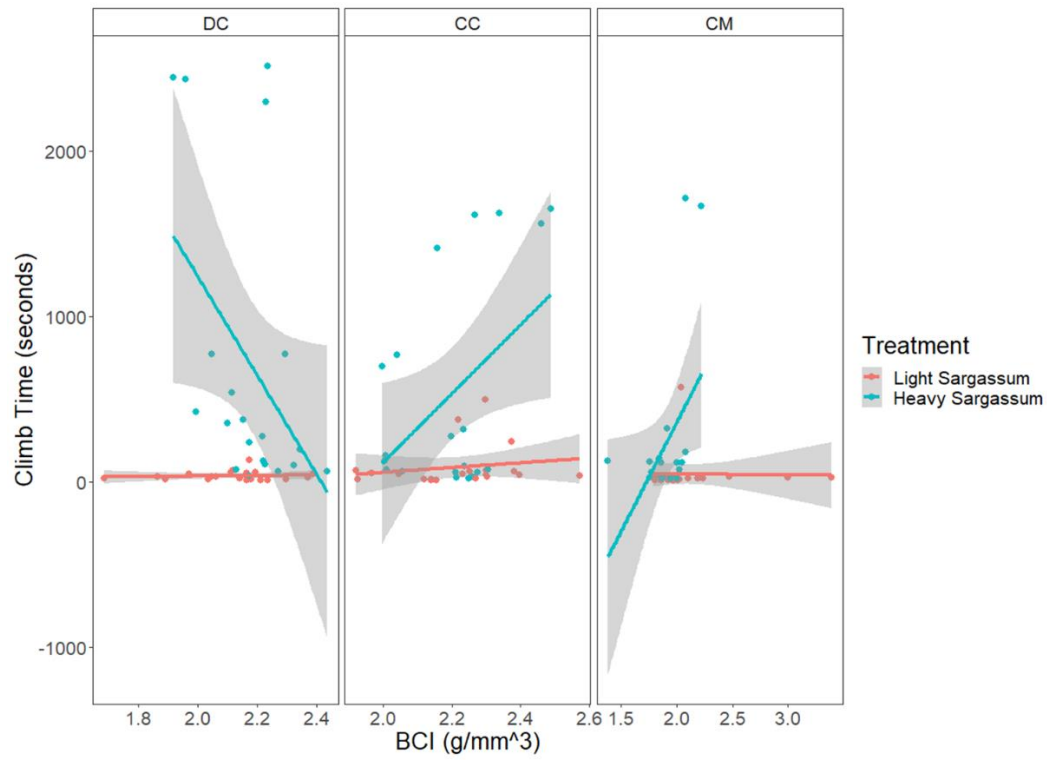


Figure 24: Correlation between body condition index (BCI) and time to climb up *Sargassum* pile for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

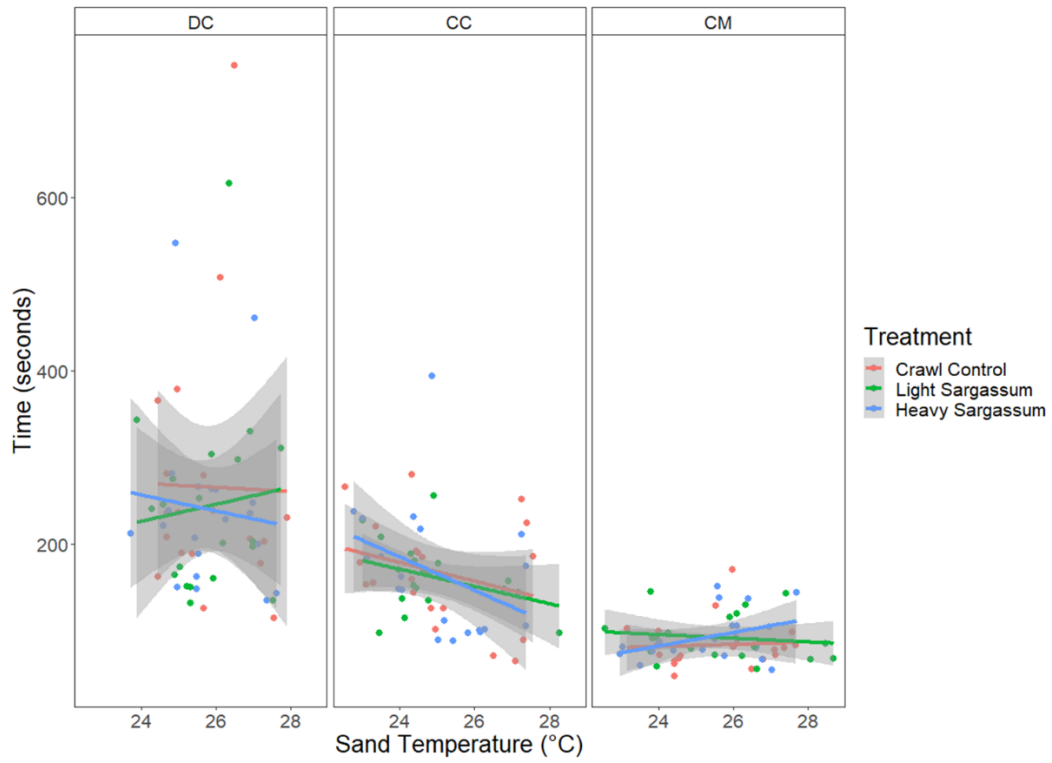


Figure 25: Correlation between sand temperature and sand time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

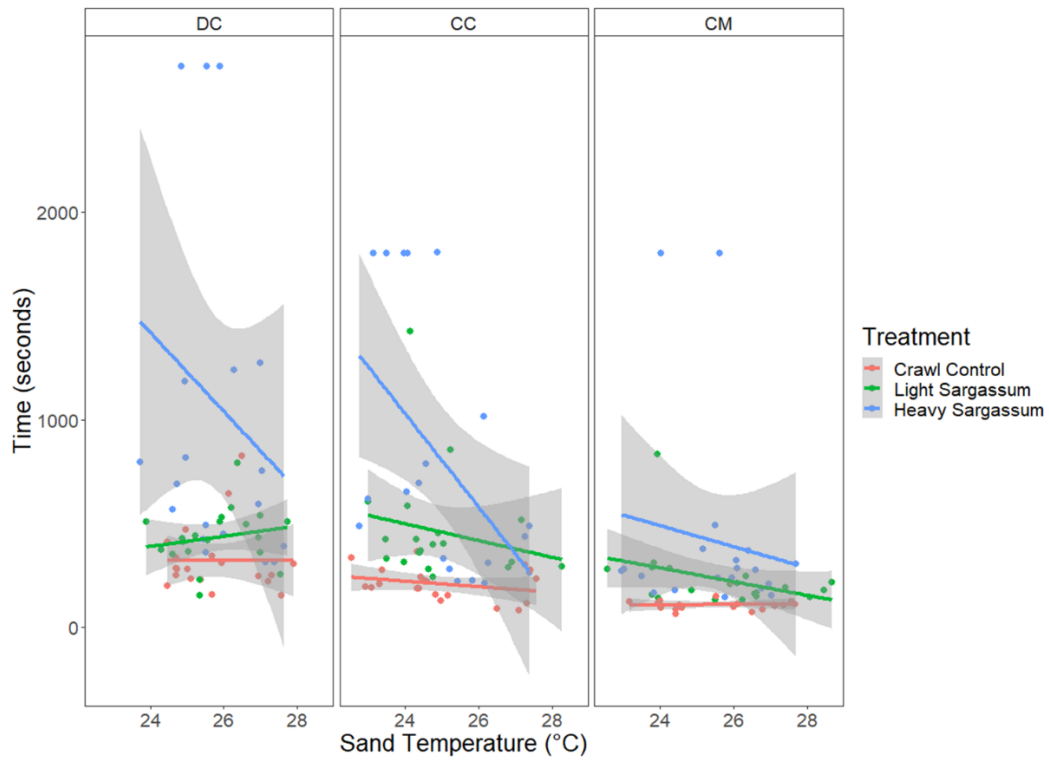


Figure 26: Correlation between sand temperature and total time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

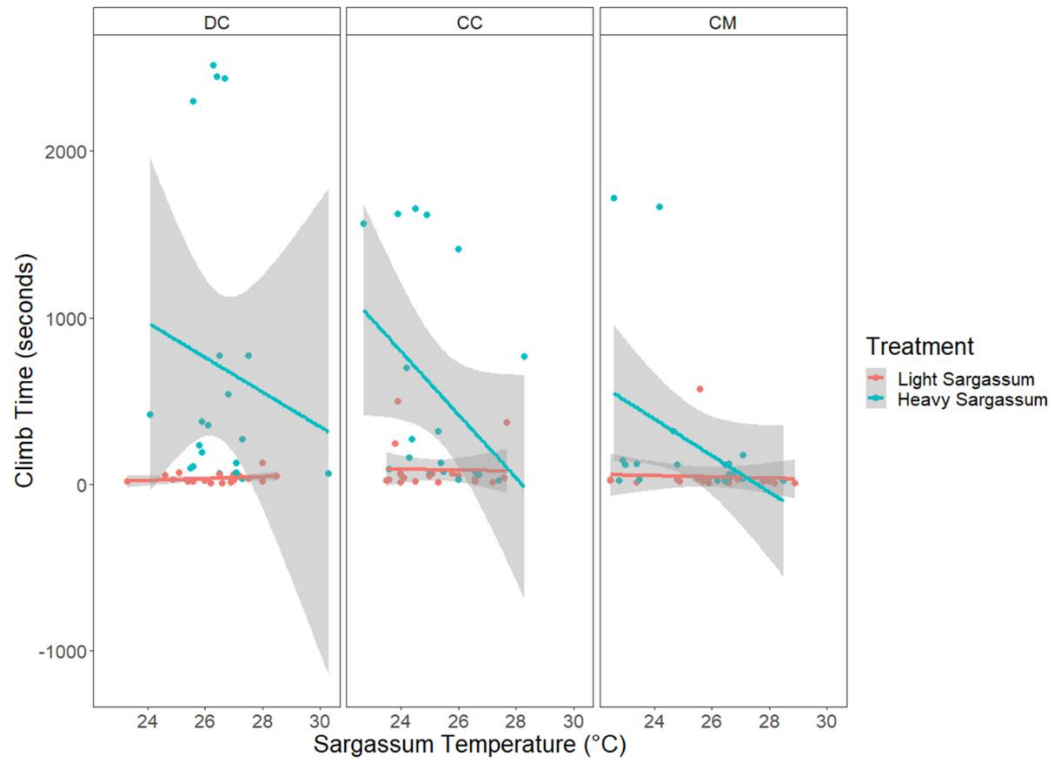


Figure 27: Correlation between *Sargassum* temperature and climb time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

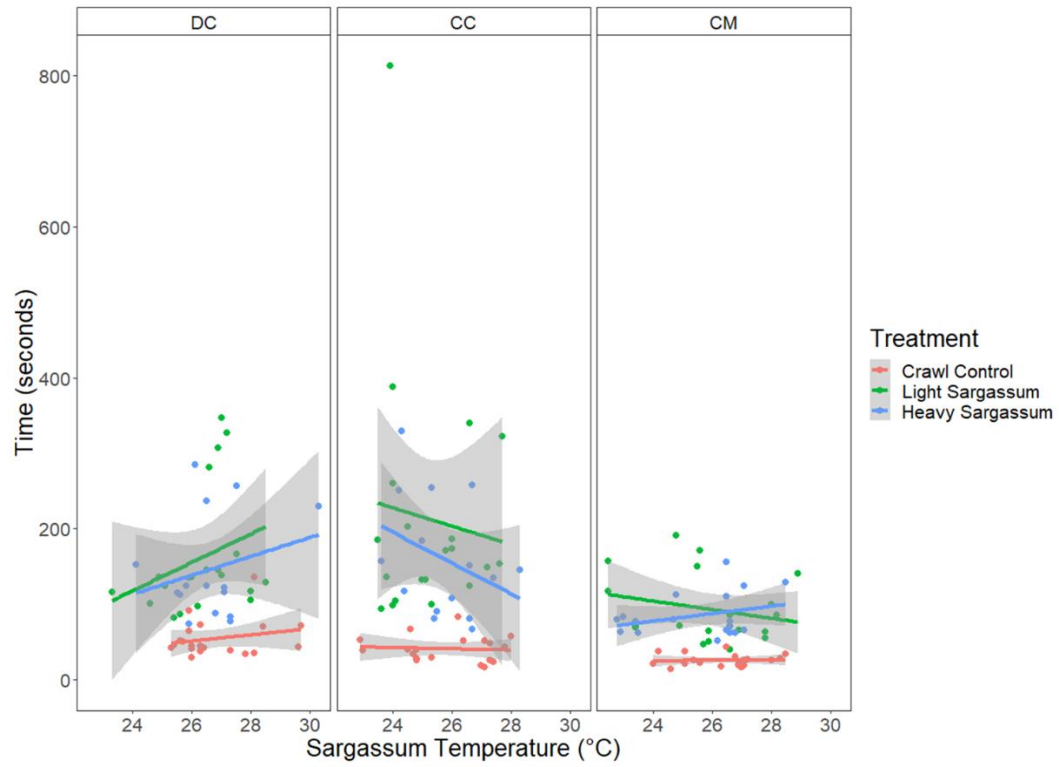


Figure 28: Correlation between *Sargassum* temperature and *Sargassum* time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

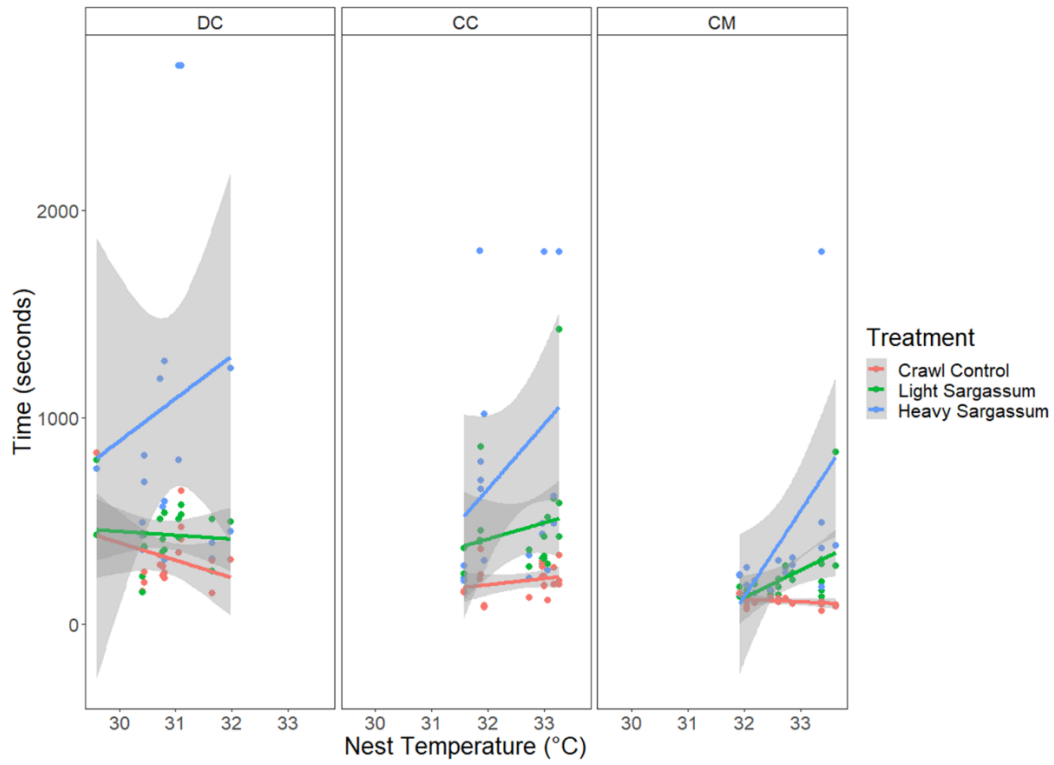


Figure 29: Correlation between overall average nest incubation temperature and total time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

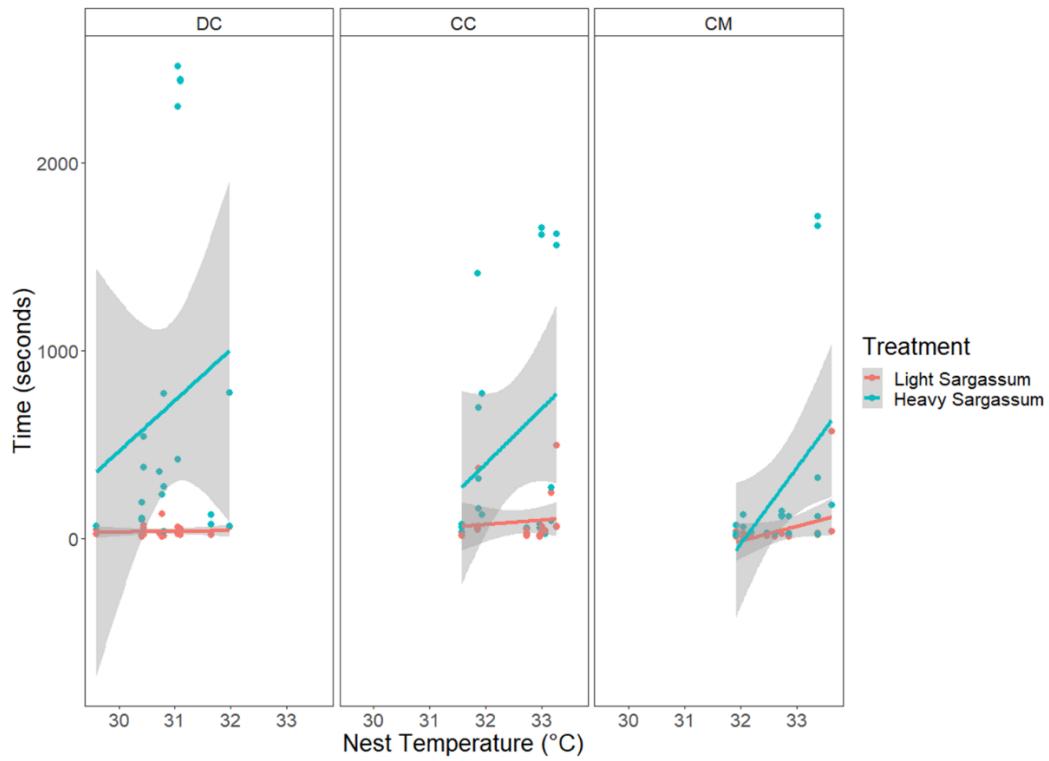


Figure 30: Correlation between overall average nest incubation temperature and *Sargassum* Climb time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

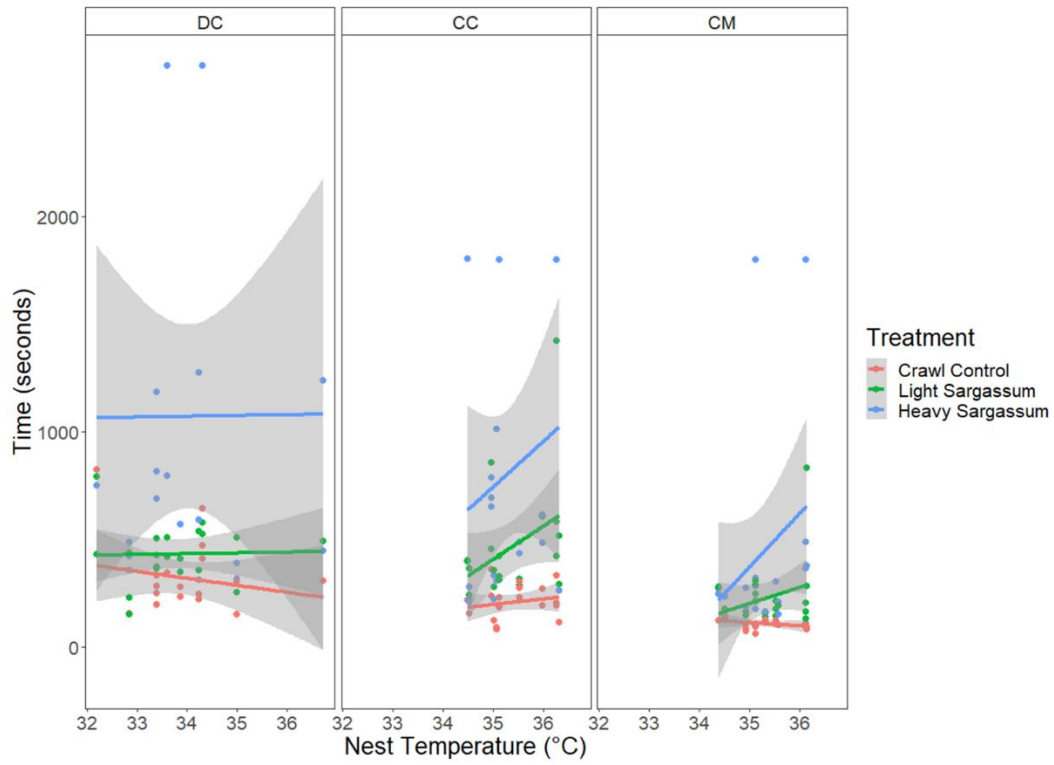


Figure 31: Correlation between single maximum nest temperature and total time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

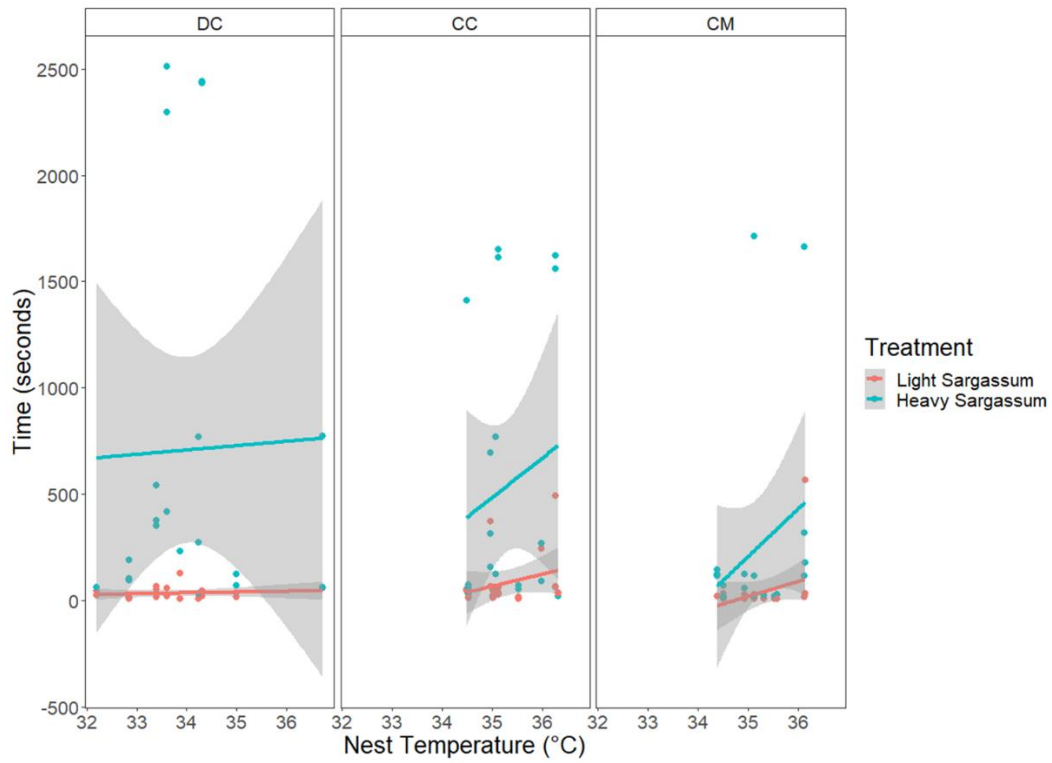


Figure 32: Correlation between single maximum nest temperature and *Sargassum* climb time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

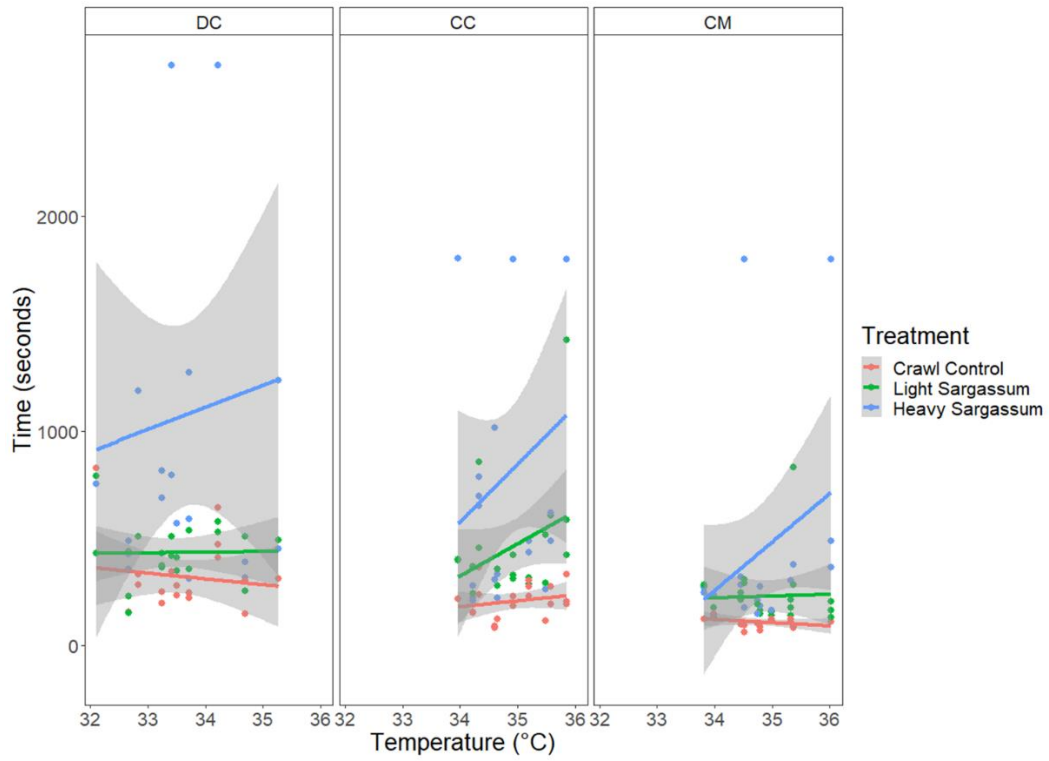


Figure 33: Correlation between highest three day average temperature and total time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

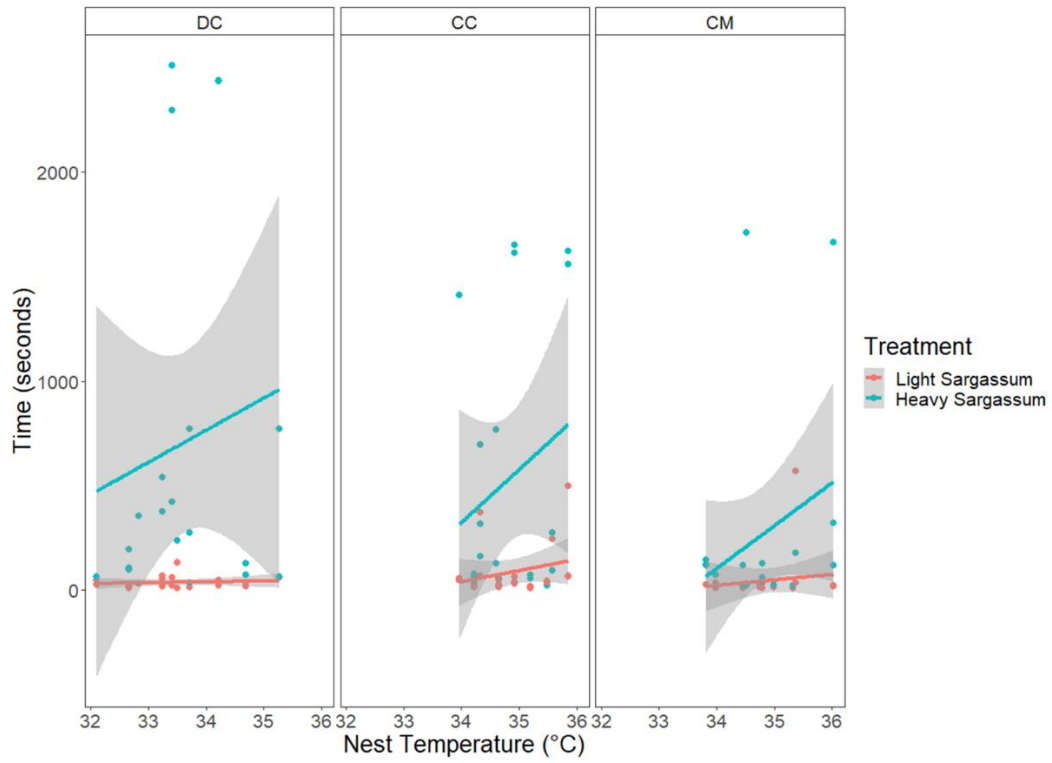


Figure 34: Correlation between highest three day average temperature and *Sargassum* climb time for each species in each treatment. Line shows the linear model, grey area is the 95% confidence interval.

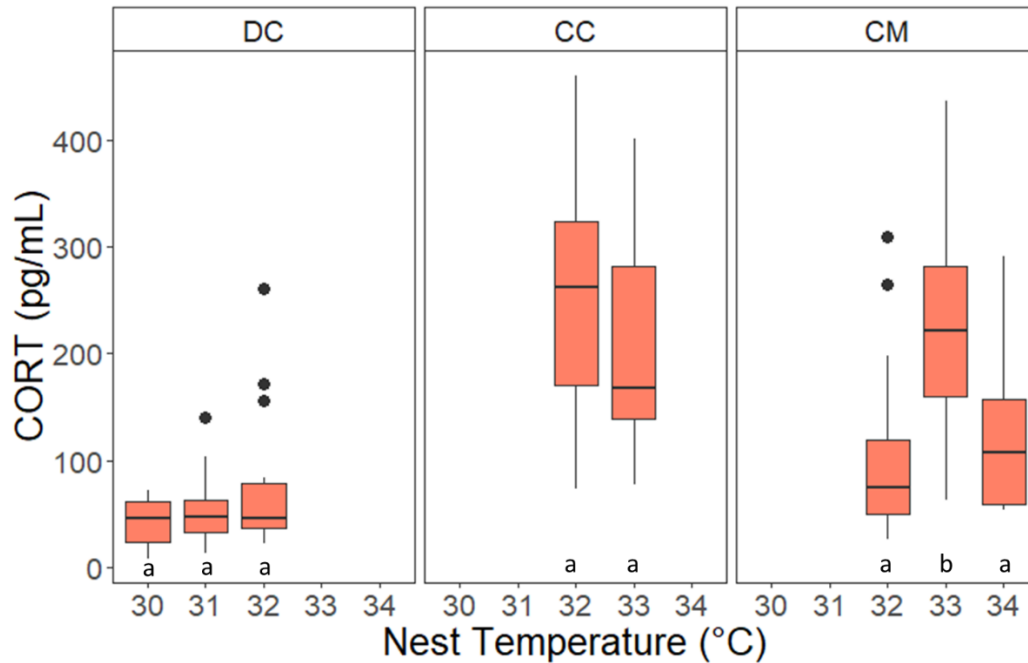


Figure 35: Blood plasma corticosterone concentrations for overall average nest incubation temperatures. The bottom line of the box represents the 25th percentile, the line in the middle of the box is the median, and the top line of the box is the 75th percentile. Error bars show the 10th and 90th percentile and outliers are indicated as dots. Significance shown using letters, boxes with different letters have significantly different medians. Significance only identified within each species, not between species.

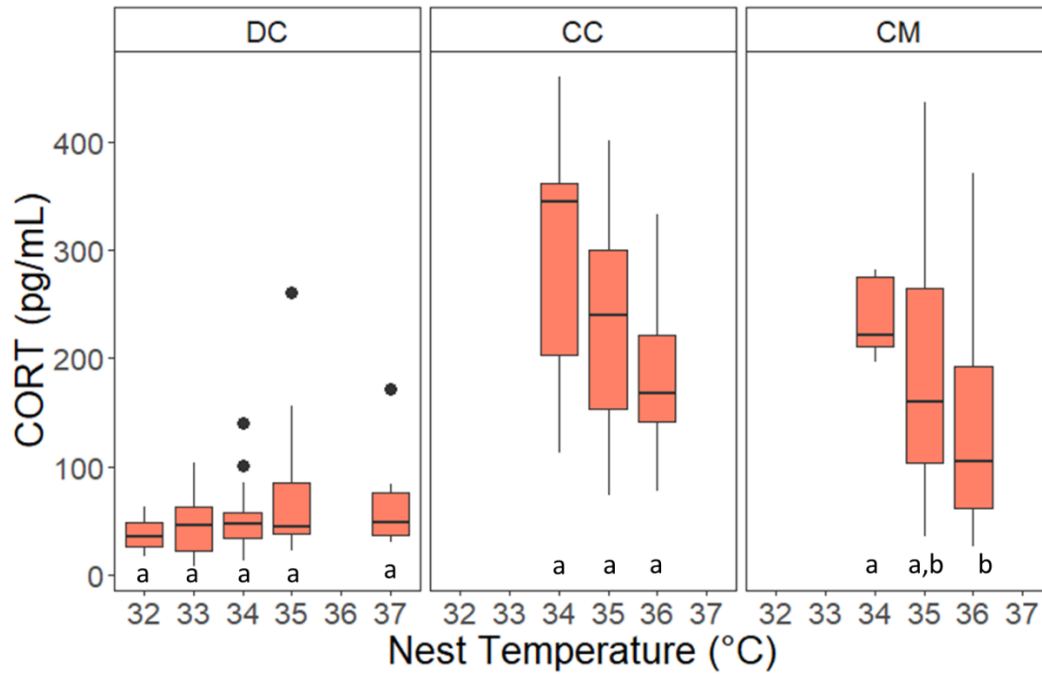


Figure 36: Blood plasma corticosterone concentrations for maximum nest incubation temperatures. The bottom line of the box represents the 25th percentile, the line in the middle of the box is the median, and the top line of the box is the 75th percentile. Error bars show the 10th and 90th percentile and outliers are indicated as dots. Significance shown using letters, boxes with different letters have significantly different medians. Significance only identified within each species, not between species.

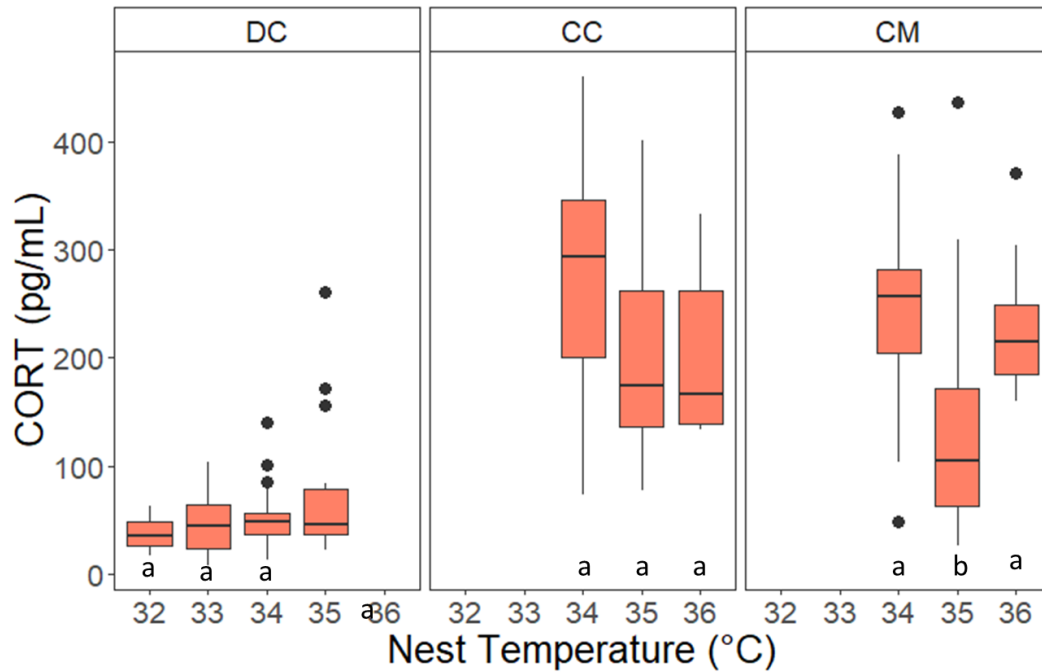


Figure 37: Blood plasma corticosterone concentrations for three-day maximum mean nest incubation temperatures. The bottom line of the box represents the 25th percentile, the line in the middle of the box is the median, and the top line of the box is the 75th percentile. Error bars show the 10th and 90th percentile and outliers are indicated as dots. Significance shown using letters, boxes with different letters have significantly different medians. Significance only identified within each species, not between species.

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